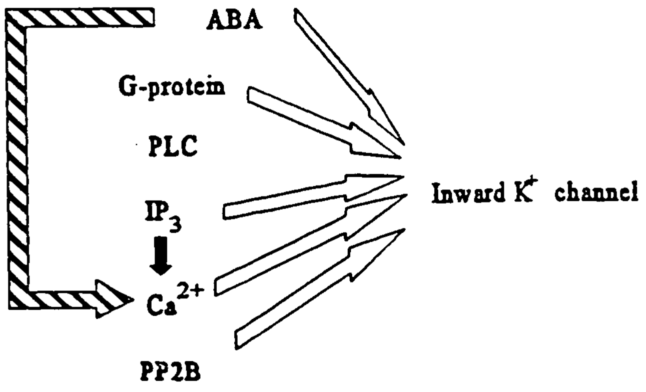
09/509738

09509738.052400

FIG. 1


ABA
G-protein
PLC
IP3
Ca2+
PP2B
Inward K+ channel

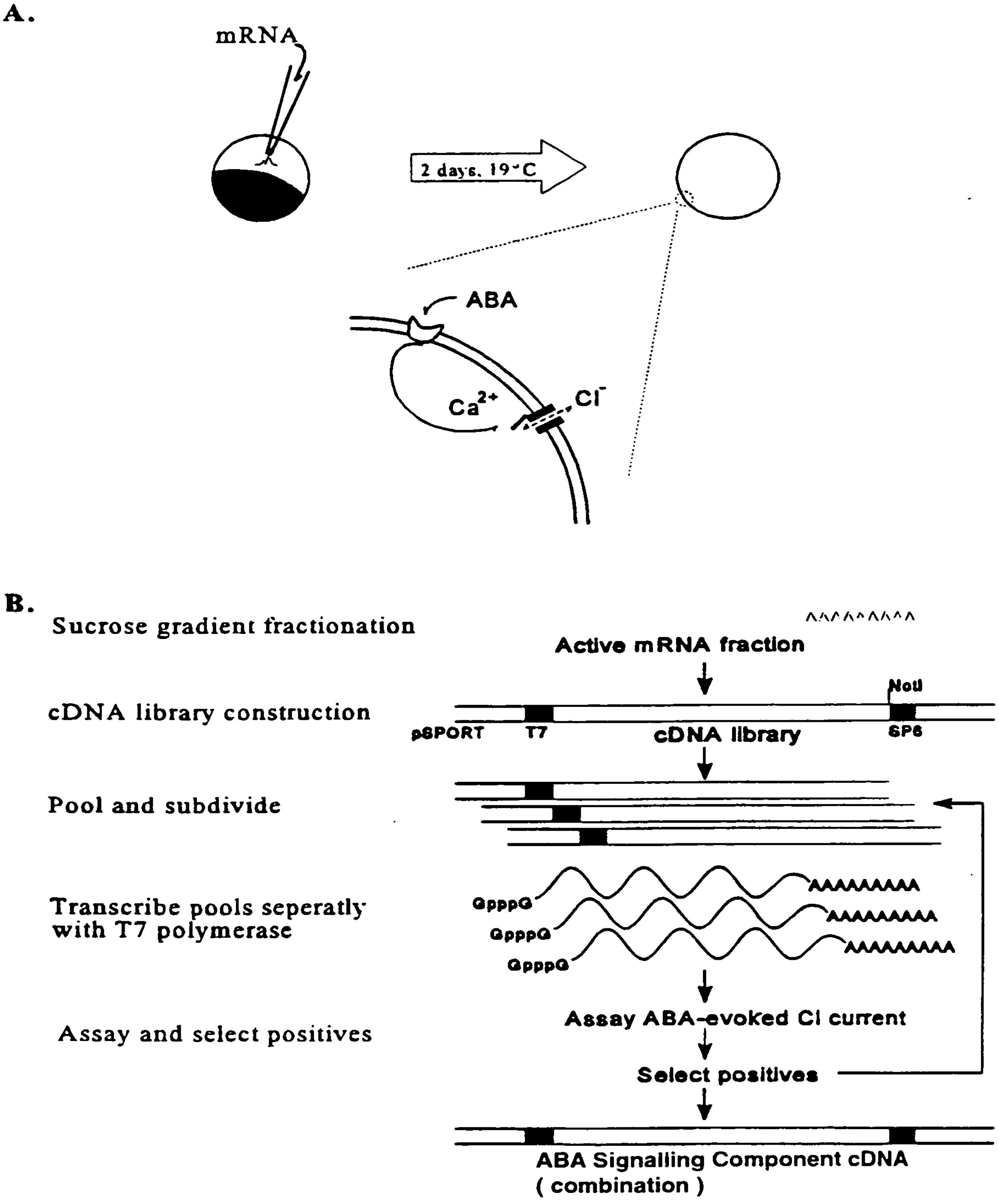
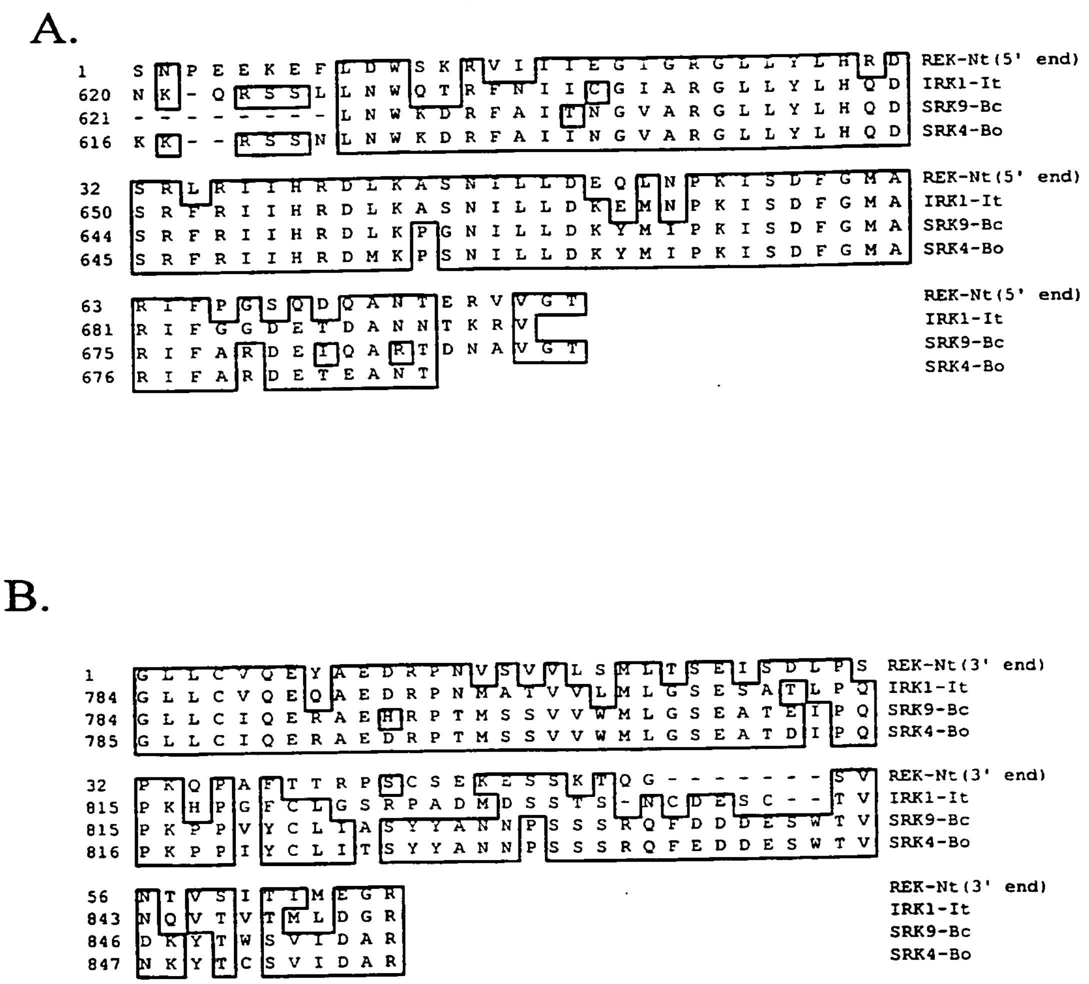

A.
mRNA
2 days, 19°C
ABA
Ca^{2+}
Cl^-
B.
Sucrose gradient fractionation
cDNA library construction
Pool and subdivide
Transcribe pools seperatly with T7 polymerase
Assay and select positives
Active mRNA fraction
NotI
pSPORT
T7
cDNA library
SP6
GpppG
AAAAAAAAA
Assay ABA-evoked Cl current
Select positives
ABA Signalling Component cDNA (combination)

FIG. 2

APPROVED O.G. FIG.
BY CLASS SUBCLASS
DRAFTSMAN

09/509738



A.

```
1     S N P E E K E F L D W S K R V I I I E G I G R G L L Y L H R D   REK-Nt(5' end)
620   N K - Q R S S L L N W Q T R F N I I C G I A R G L L Y L H Q D   IRK1-It
621   - - - - - - - - L N W K D R F A I T N G V A R G L L Y L H Q D   SRK9-Bc
616   K K - - R S S N L N W K D R F A I I N G V A R G L L Y L H Q D   SRK4-Bo

32    S R L R I I H R D L K A S N I L L D E Q L N P K I S D F G M A   REK-Nt(5' end)
650   S R F R I I H R D L K A S N I L L D K E M N P K I S D F G M A   IRK1-It
644   S R F R I I H R D L K P G N I L L D K Y M I P K I S D F G M A   SRK9-Bc
645   S R F R I I H R D M K P S N I L L D K Y M I P K I S D F G M A   SRK4-Bo

63    R I F P G S Q D Q A N T E R V V G T                             REK-Nt(5' end)
681   R I F G G D E T D A N N T K R V                                 IRK1-It
675   R I F A R D E I Q A R T D N A V G T                             SRK9-Bc
676   R I F A R D E T E A N T                                         SRK4-Bo
```

B.

```
1     G L L C V Q E Y A E D R P N V S V V L S M L T S E I S D L P S   REK-Nt(3' end)
784   G L L C V Q E Q A E D R P N M A T V V L M L G S E S A T L P Q   IRK1-It
784   G L L C I Q E R A E H R P T M S S V V W M L G S E A T E I P Q   SRK9-Bc
785   G L L C I Q E R A E D R P T M S S V V W M L G S E A T D I P Q   SRK4-Bo

32    P K Q P A F T T R P S C S E K E S S K T Q G - - - - - - - - S V   REK-Nt(3' end)
815   P K H P G F C L G S R P A D M D S S T S - N C D E S C - - T V   IRK1-It
815   P K P P V Y C L I A S Y Y A N N P S S S R Q F D D D E S W T V   SRK9-Bc
816   P K P P I Y C L I T S Y Y A N N P S S S R Q F E D D E S W T V   SRK4-Bo

56    N T V S I T I M E G R                                           REK-Nt(3' end)
843   N Q V T V T M L D G R                                           IRK1-It
846   D K Y T W S V I D A R                                           SRK9-Bc
847   N K Y T C S V I D A R                                           SRK4-Bo
```

FIG. 3

09509738.032300

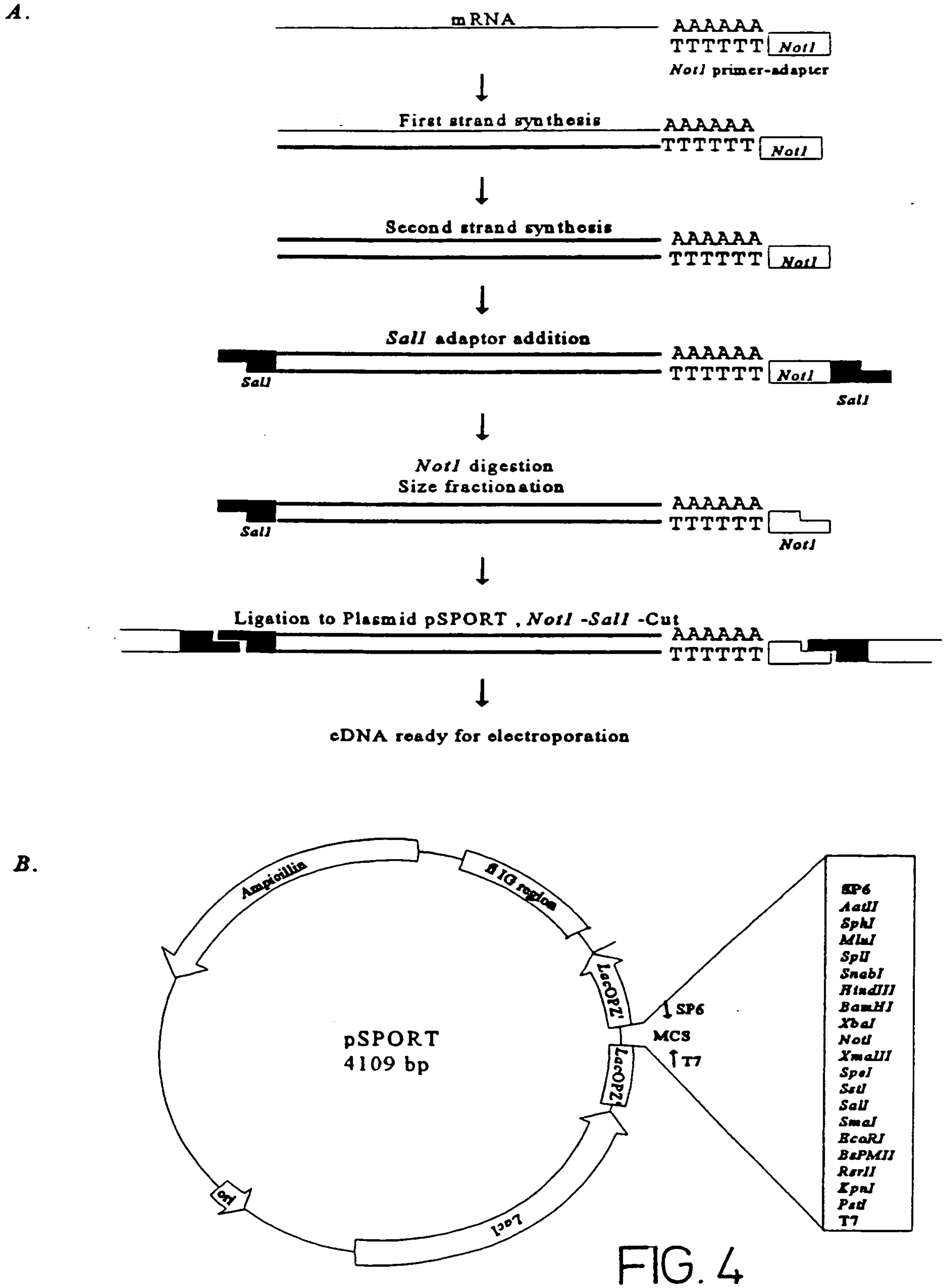

A.
mRNA
AAAAAA
TTTTTT NotI
NotI primer-adapter
First strand synthesis
AAAAAA
TTTTTT NotI
Second strand synthesis
AAAAAA
TTTTTT NotI
SalI adaptor addition
SalI
AAAAAA
TTTTTT NotI
SalI
NotI digestion
Size fractionation
SalI
AAAAAA
TTTTTT
NotI
Ligation to Plasmid pSPORT , NotI -SalI -Cut
AAAAAA
TTTTTT
cDNA ready for electroporation
B.
Ampicillin
f1 IG region
LacOPZ
LacOPZ
ori
LacI
pSPORT
4109 bp
SP6
MCS
T7
SP6
AatII
SphI
MluI
SplI
SnabI
HindIII
BamHI
XbaI
NotI
XmaIII
SpeI
SstI
SalI
SmaI
EcoRI
BsPMII
RsrII
KpnI
PstI
T7

FIG. 4

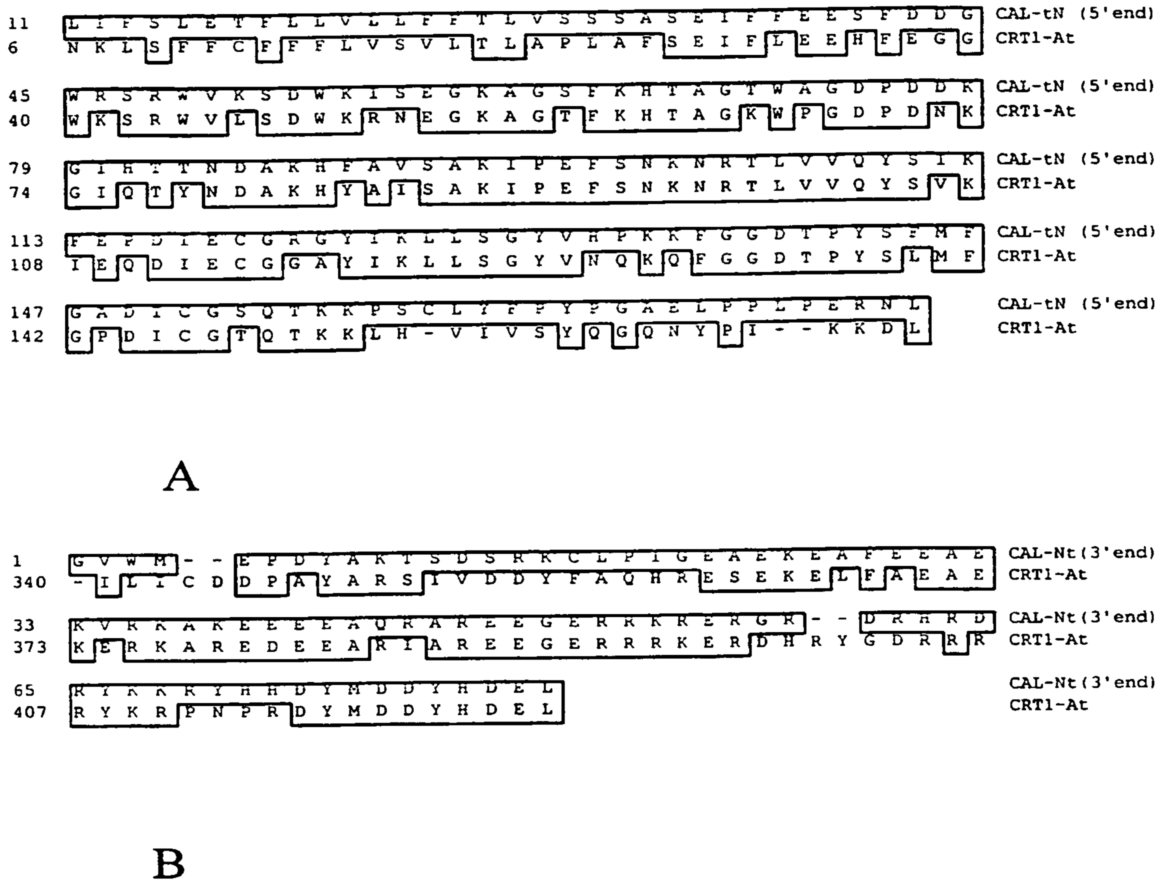

11 L I F S L E T F L L V L L F F T L V S S S A S E I F F E E S F D D G CAL-tN (5'end)
6 N K L S F F C F F F L V S V L T L A P L A F S E I F L E E H F E G G CRT1-At
45 W R S R W V K S D W K I S E G K A G S F K H T A G T W A G D P D D K CAL-tN (5'end)
40 W K S R W V L S D W K R N E G K A G T F K H T A G K W P G D P D N K CRT1-At
79 G I H T T N D A K H F A V S A K I P E F S N K N R T L V V Q Y S I K CAL-tN (5'end)
74 G I Q T Y N D A K H Y A I S A K I P E F S N K N R T L V V Q Y S V K CRT1-At
113 F E P D I E C G R G Y I K L L S G Y V H P K K F G G D T P Y S F M F CAL-tN (5'end)
108 I E Q D I E C G G A Y I K L L S G Y V N Q K Q F G G D T P Y S L M F CRT1-At
147 G A D I C G S Q T K K P S C L Y F F Y F G A E L P P L P E R N L CAL-tN (5'end)
142 G P D I C G T Q T K K L H - V I V S Y Q G Q N Y P I - - K K D L CRT1-At
A
1 G V W M - - E P D Y A K T S D S R K C L P I G E A E K E A F E E A E CAL-Nt(3'end)
340 - I L I C D D P A Y A R S I V D D Y F A Q H R E S E K E L F A E A E CRT1-At
33 K V R K A K E E E E A Q R A R E E G E R K K R E R G R - - D R H R D CAL-Nt(3'end)
373 K E R K A R E D E E A R I A R E E G E R R R K E R D H R Y G D R R R CRT1-At
65 R Y K K R Y H H D Y M D D Y H D E L CAL-Nt(3'end)
407 R Y K R P N P R D Y M D D Y H D E L CRT1-At
B

FIG. 7

APPROVED BY DRAFTSMAN | O.G. FIG. | CLASS WO | SUBCLASS 99/16880

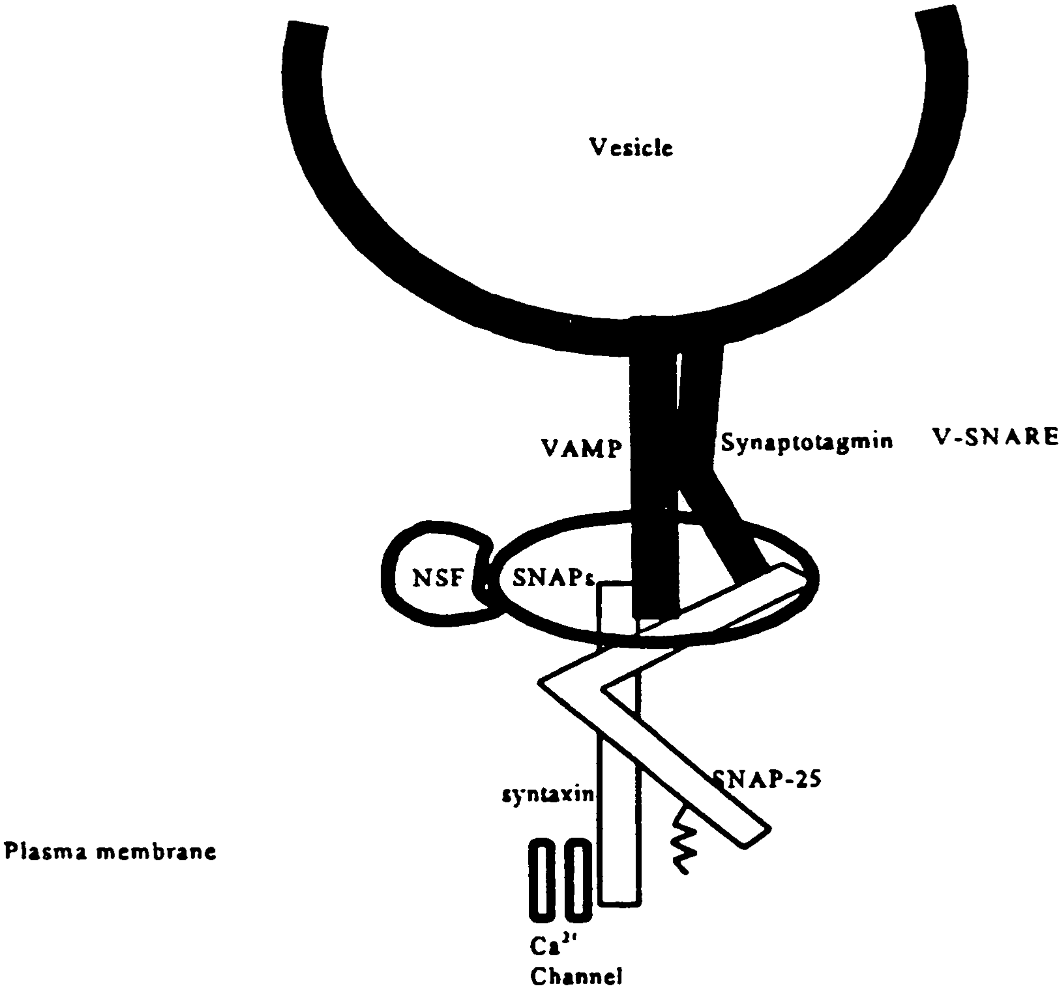
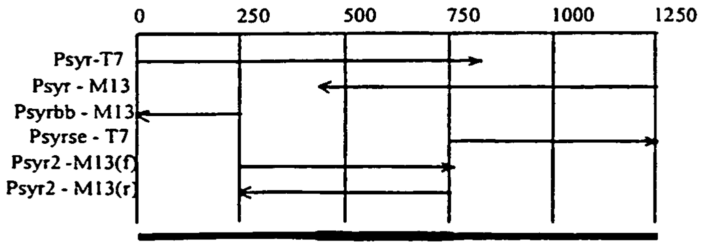

Vesicle
VAMP
Synaptotagmin
V-SNARE
NSF
SNAPs
syntaxin
SNAP-25
Plasma membrane
Ca^{2+}
Channel

FIG. 8

```
CCAAATCCCATCTCAAAATGAATGATCTATTTTCAGGATCTTTCTCTCGTTTCAGAGCTG      60
                 M  N  D  L  F  S  G  S  F  S  R  F  R  A
ACGATCAATCGGACTCTCACGCCATAGAAATGGGAGACATTACTGGCGGAGTCAATCTCG      120
D  D  Q  S  D  S  H  A  I  E  M  G  D  I  T  G  G  V  N  L
ACAAATTCTTCGAAGATGTTGAAGCCATTAAAGACGAACTCAAAGGCCTCGAGAAAATCT      180
D  K  F  F  E  D  V  E  A  I  K  D  E  L  K  G  L  E  K  I
ATTCCCAACTCCAATCTTCCCATGAAAAAAGCAAGACTCTTCACAACGCTAAAGCCGTTA      240
Y  S  Q  L  Q  S  S  H  E  K  S  K  T  L  H  N  A  K  A  V
AAGATCTAAGATCCAACATGGATAATGACGTTTCCATGGCATTGAAGAAAGCCAAATTCA      300
K  D  L  R  S  N  M  D  N  D  V  S  M  A  L  K  K  A  K  F
TCAAAGTTCGTCTCGAAGCCTTAGACAGATCAAATGCAGCGAATCGAAGCCTCCCTGGAT      360
I  K  V  R  L  E  A  L  D  R  S  N  A  A  N  R  S  L  P  G
GTGGACCCGGAAGTTCATCTGACAGGACGAGAACTTCAGTTGTGAACGGATTAAGGAAGA      420
C  G  P  G  S  S  S  D  R  T  R  T  S  V  V  N  G  L  R  K
AACTTCAAGAGTCAATGAATCAGTTCAACGAGCTAAGGCAAAAGATGGCATCTGAATATA      480
K  L  Q  E  S  M  N  Q  F  N  E  L  R  Q  K  M  A  S  E  Y
GGGAAACAGTTCAACGACGATATTATACCGTCACAGGAGAAAATCCTGATGAAGCAGTTC      540
R  E  T  V  Q  R  R  Y  Y  T  V  T  G  E  N  P  D  E  A  V
TTGATACACTCATATCTACAGGTCAAAGTGAGACGTTCTTGCAAAAGGCAATTCAAGAGC      600
L  D  T  L  I  S  T  G  Q  S  E  T  F  L  Q  K  A  I  Q  E
AAGGGAGAGGACAAGTGATGGATACAGTTATGGAAATTCAAGAAAGGCATGAAGCTGTGA      660
Q  G  R  G  Q  V  M  D  T  V  M  E  I  Q  E  R  H  E  A  V
AGGAATTGGAGAGGAATTTGAAAGAATTGCATCAAGTATTCTTGGACATGGCTGTTTTGG      720
K  E  L  E  R  N  L  K  E  L  H  Q  V  F  L  D  M  A  V  L
TTGAAAGTCAAGGAGCTCAACTTGATGATATTGAGAGCCAAGTGAATAGGGCTAATTCCT      780
V  E  S  Q  G  A  Q  L  D  D  I  E  S  Q  V  N  R  A  N  S
TCGTTAGAGGGGGTGCTCAGCAACTGCAAGTGGCAAGGAAGCACCAGAAGAACACTAGAA      840
F  V  R  G  G  A  Q  Q  L  Q  V  A  R  K  H  Q  K  N  T  R
AATGGACTTGTTTTGCTATTATTCTTCTGCTTATCATCATTTTGGTGGTGGTTCTTTCTA      900
K  W  T  C  F  A  I  I  L  L  L  I  I  I  L  V  V  V  L  S
TTCAGCCATGGAAAAAATGAGAATTTGTCTATGGTCAAAGGTCTTCTGGTGGACCCCTTC      960
I  Q  P  W  K  K  .
AATGTTTTGAATATTCTAAATTTTTATATTTTATTATTTTAGCCATGCTTATTATTTTGT      1020
GTTATTTTGGATTTTTTTTTTGTTTTTAATGTGGGGAAGAGTAAACTGGATGGGGGTCCA      1080
TGTGCTATTTAGAGAAATACTTGGGAGTTCTCTTTTTGTAATTATTGCTGTATTTAGAGT      1140
ATAATTCTTTTTCTATATTGTTGGCAGGTTAATTTGTTTGTTTGATTATATTCTCATTTA      1200
GATTT                                                             1205
```

A



B

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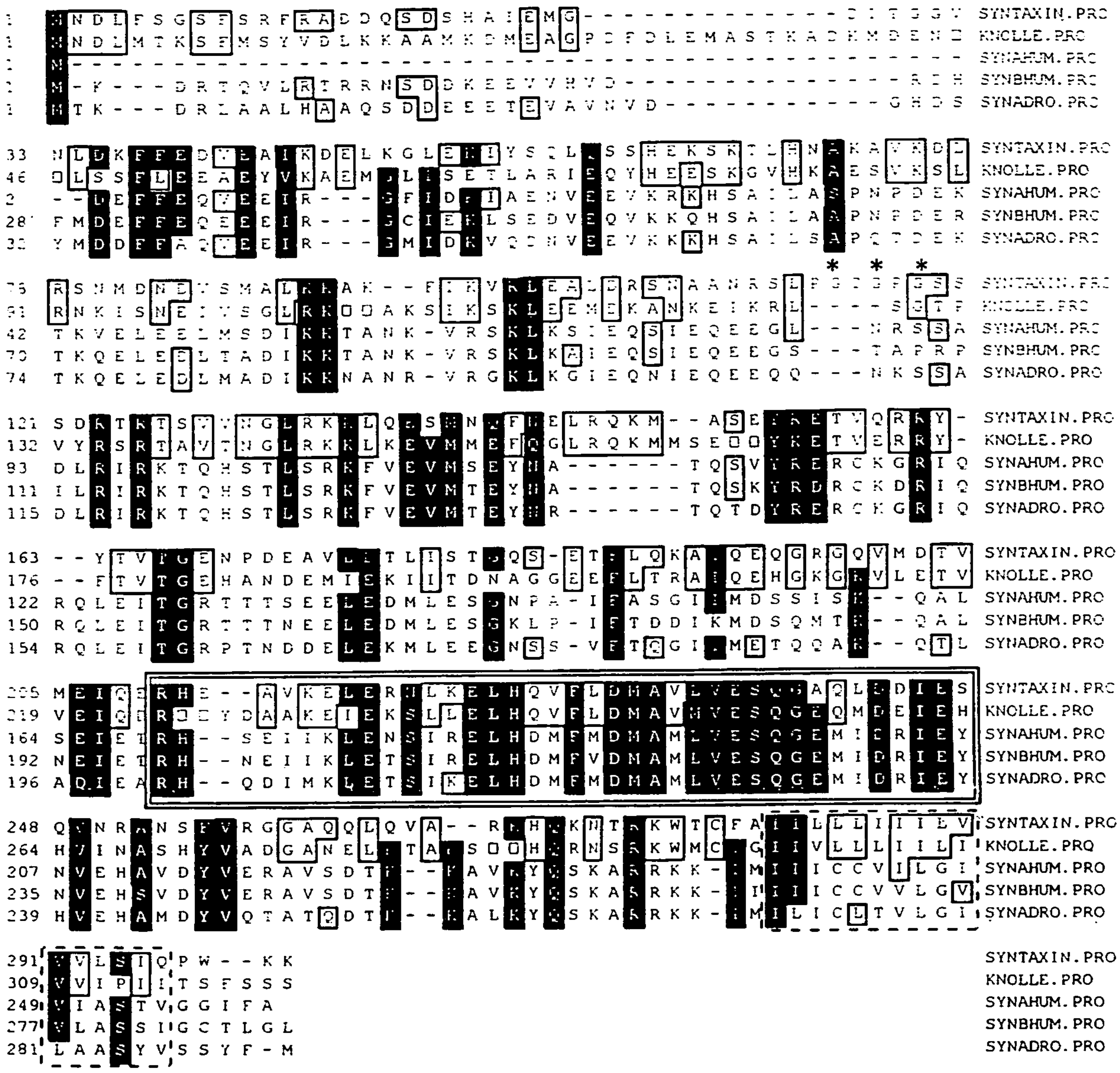
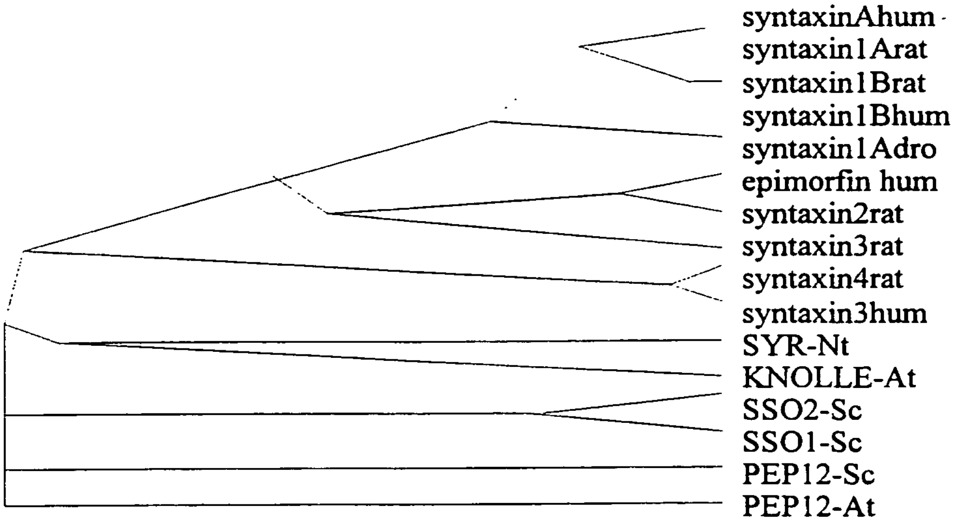

SYNTAXIN.PRO
KNOLLE.PRO
SYNAHUM.PRO
SYNBHUM.PRO
SYNADRO.PRO

FIG.10

09/509738

FIG 11


syntaxinAhum
syntaxin1Arat
syntaxin1Brat
syntaxin1Bhum
syntaxin1Adro
epimorfin hum
syntaxin2rat
syntaxin3rat
syntaxin4rat
syntaxin3hum
SYR-Nt
KNOLLE-At
SSO2-Sc
SSO1-Sc
PEP12-Sc
PEP12-At

09509738 .052400

09/509738

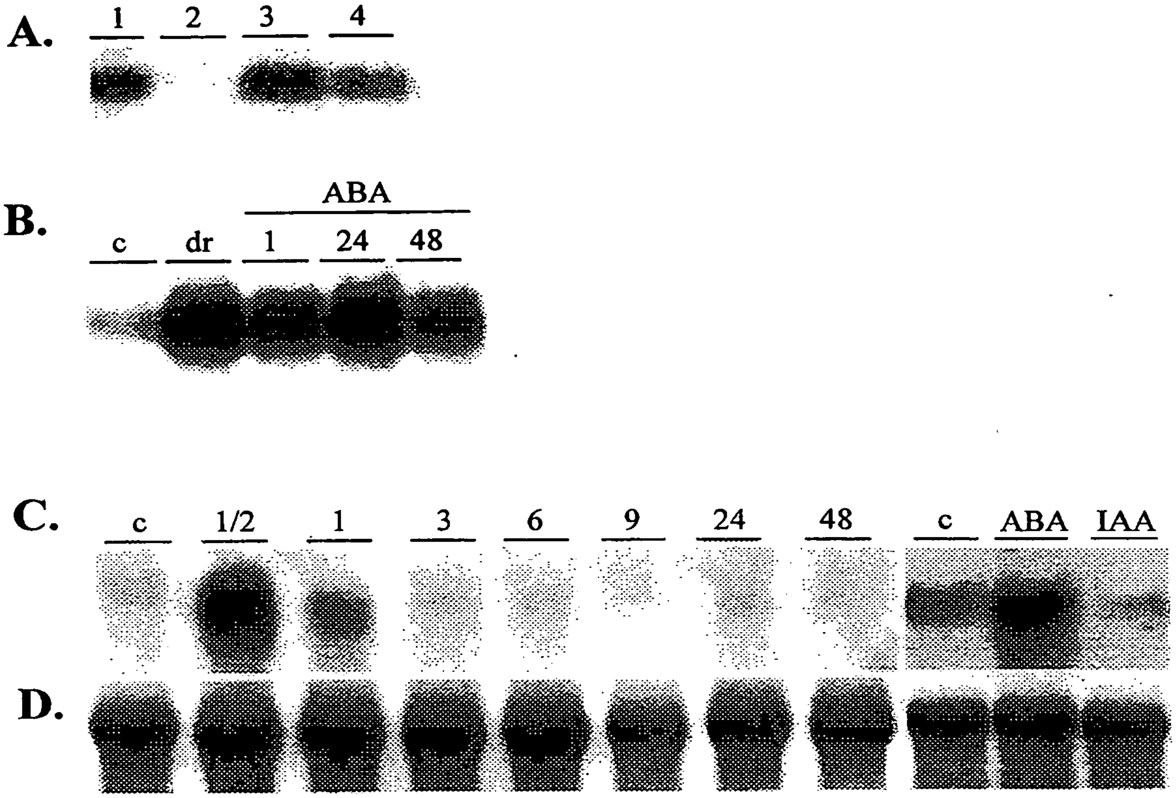

A.
1
2
3
4
B.
ABA
c
dr
1
24
48
C.
c
1/2
1
3
6
9
24
48
c
ABA
IAA
D.

FIG. 12

09509738 .052400

09509738 . 052500

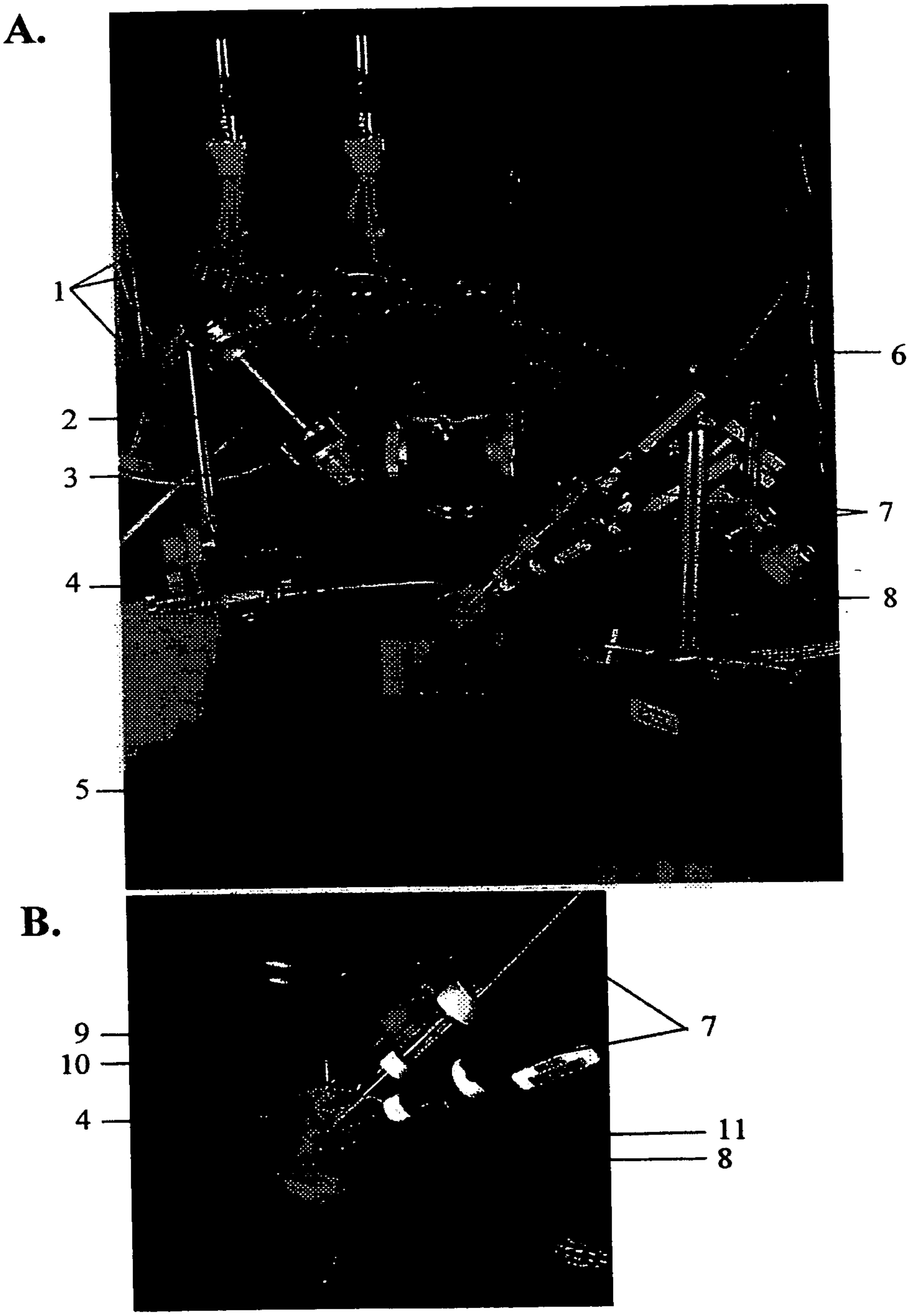

A.
1
2
3
4
5
6
7
8
B.
9
10
4
7
11
8

FIG. 13

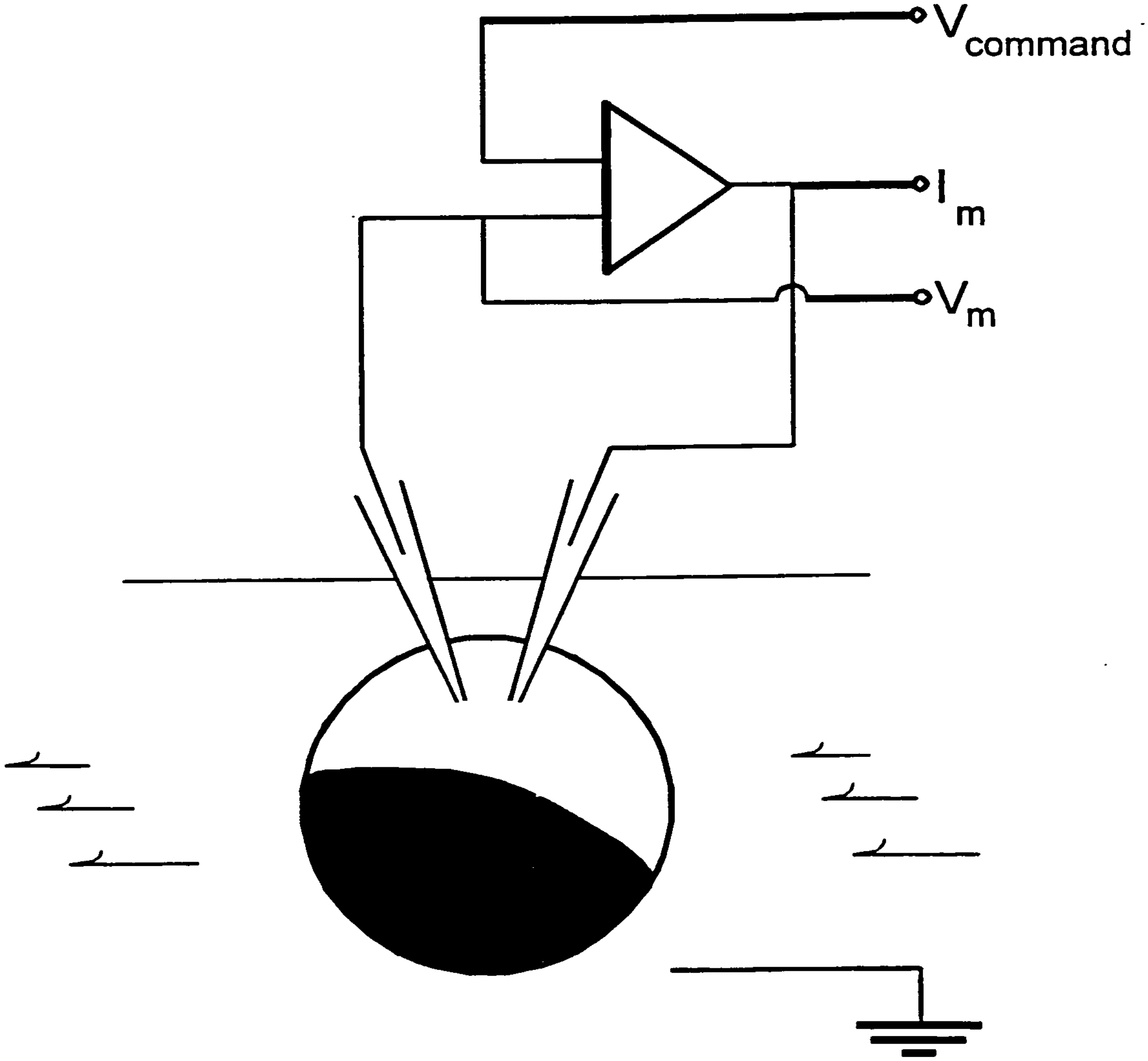


FIG. 14

09509738 .052400

09509738.052400

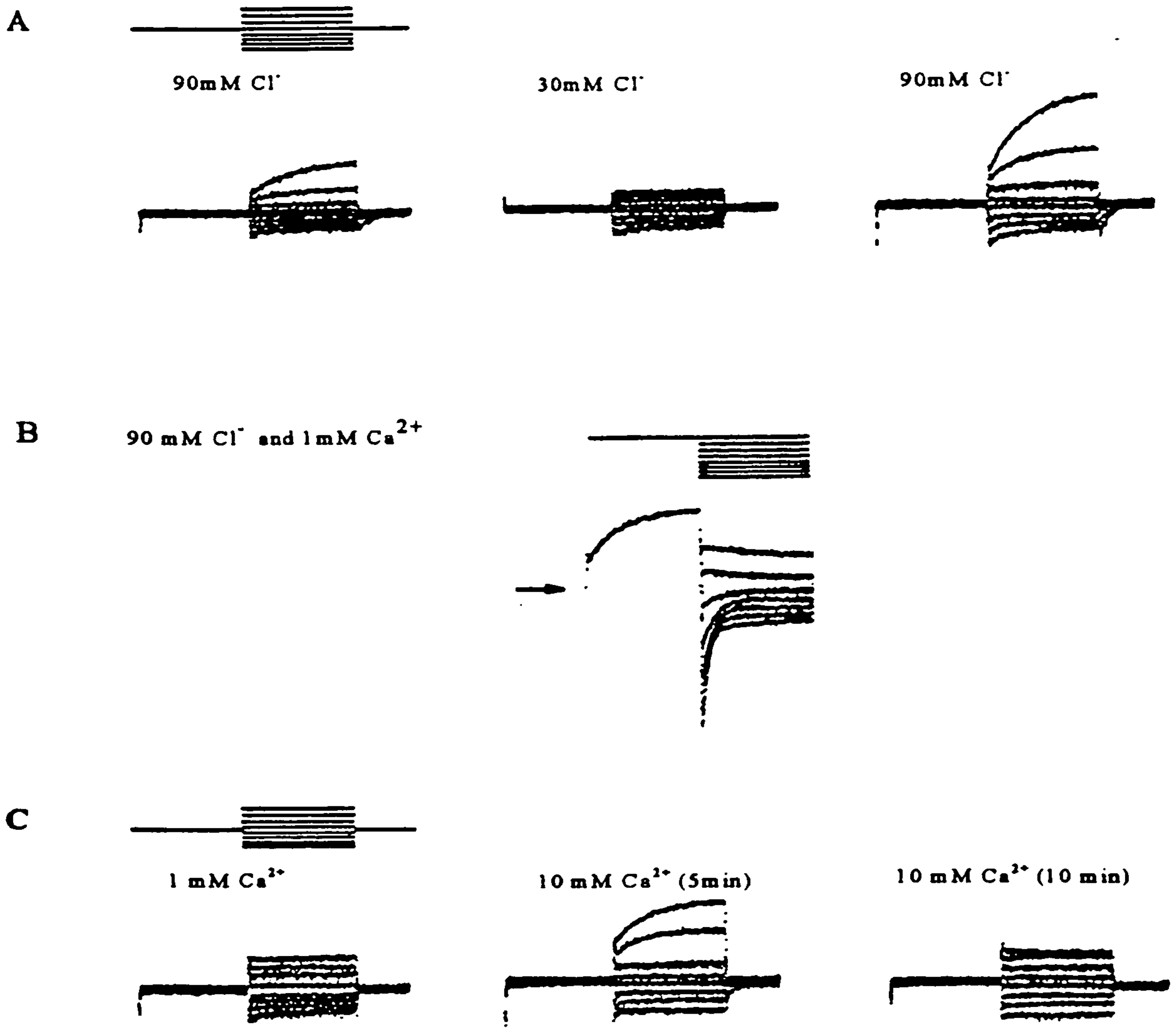


FIG.15

FIG 5

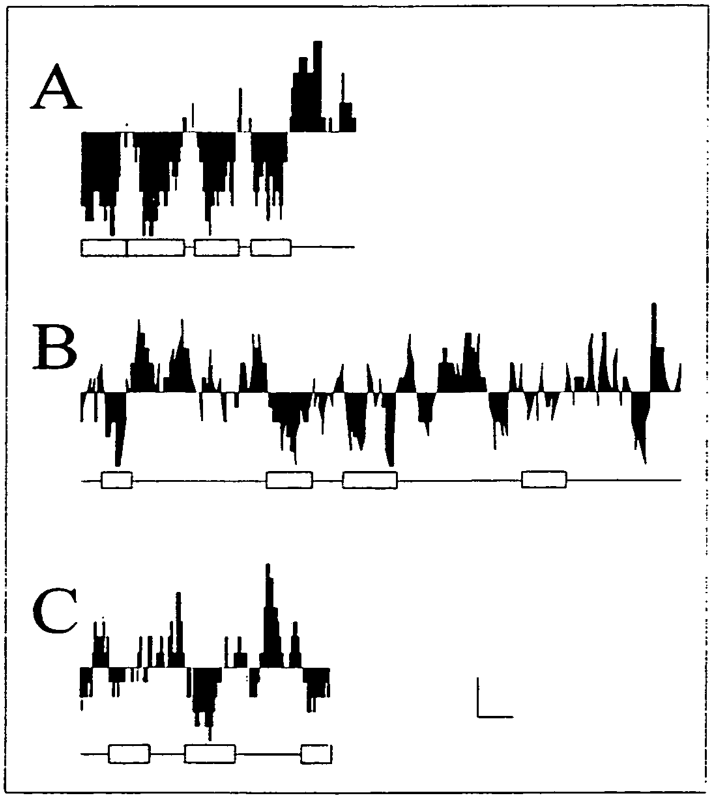
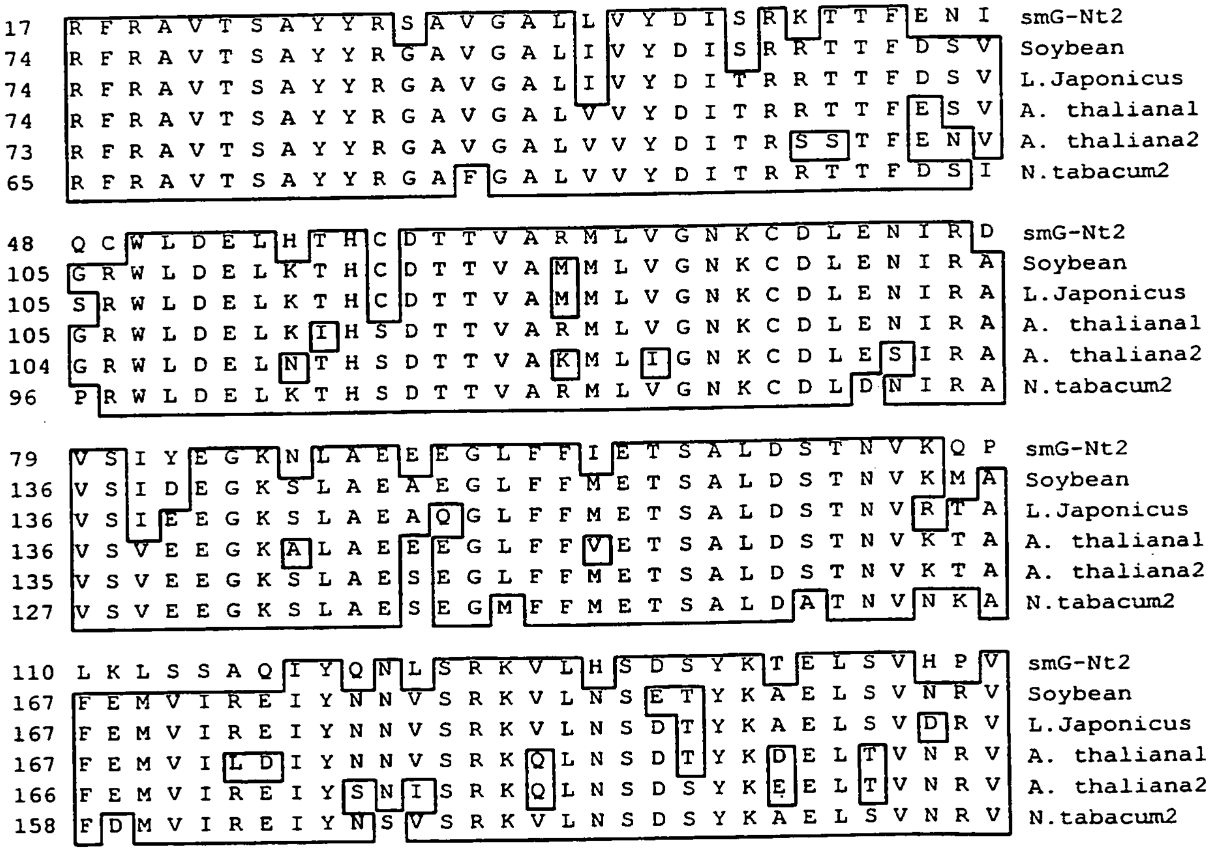


FIG. 6



```
 17 R F R A V T S A Y Y R S A V G A L L V Y D I S R K T T F E N I smG-Nt2
 74 R F R A V T S A Y Y R G A V G A L I V Y D I S R R T T F D S V Soybean
 74 R F R A V T S A Y Y R G A V G A L I V Y D I T R R T T F D S V L.Japonicus
 74 R F R A V T S A Y Y R G A V G A L V V Y D I T R R T T F E S V A. thaliana1
 73 R F R A V T S A Y Y R G A V G A L V V Y D I T R S S T F E N V A. thaliana2
 65 R F R A V T S A Y Y R G A F G A L V V Y D I T R R T T F D S I N.tabacum2

 48 Q C W L D E L H T H C D T T V A R M L V G N K C D L E N I R D smG-Nt2
105 G R W L D E L K T H C D T T V A M M L V G N K C D L E N I R A Soybean
105 S R W L D E L K T H C D T T V A M M L V G N K C D L E N I R A L.Japonicus
105 G R W L D E L K I H S D T T V A R M L V G N K C D L E N I R A A. thaliana1
104 G R W L D E L N T H S D T T V A K M L I G N K C D L E S I R A A. thaliana2
 96 P R W L D E L K T H S D T T V A R M L V G N K C D L D N I R A N.tabacum2

 79 V S I Y E G K N L A E E E G L F F I E T S A L D S T N V K Q P smG-Nt2
136 V S I D E G K S L A E A E G L F F M E T S A L D S T N V K M A Soybean
136 V S I E E G K S L A E A Q G L F F M E T S A L D S T N V R T A L.Japonicus
136 V S V E E G K A L A E E E G L F F V E T S A L D S T N V K T A A. thaliana1
135 V S V E E G K S L A E S E G L F F M E T S A L D S T N V K T A A. thaliana2
127 V S V E E G K S L A E S E G M F F M E T S A L D A T N V N K A N.tabacum2

110 L K L S S A Q I Y Q N L S R K V L H S D S Y K T E L S V H P V smG-Nt2
167 F E M V I R E I Y N N V S R K V L N S E T Y K A E L S V N R V Soybean
167 F E M V I R E I Y N N V S R K V L N S D T Y K A E L S V D R V L.Japonicus
167 F E M V I L D I Y N N V S R K Q L N S D T Y K D E L T V N R V A. thaliana1
166 F E M V I R E I Y S N I S R K Q L N S D S Y K E E L T V N R V A. thaliana2
158 F D M V I R E I Y N S V S R K V L N S D S Y K A E L S V N R V N.tabacum2
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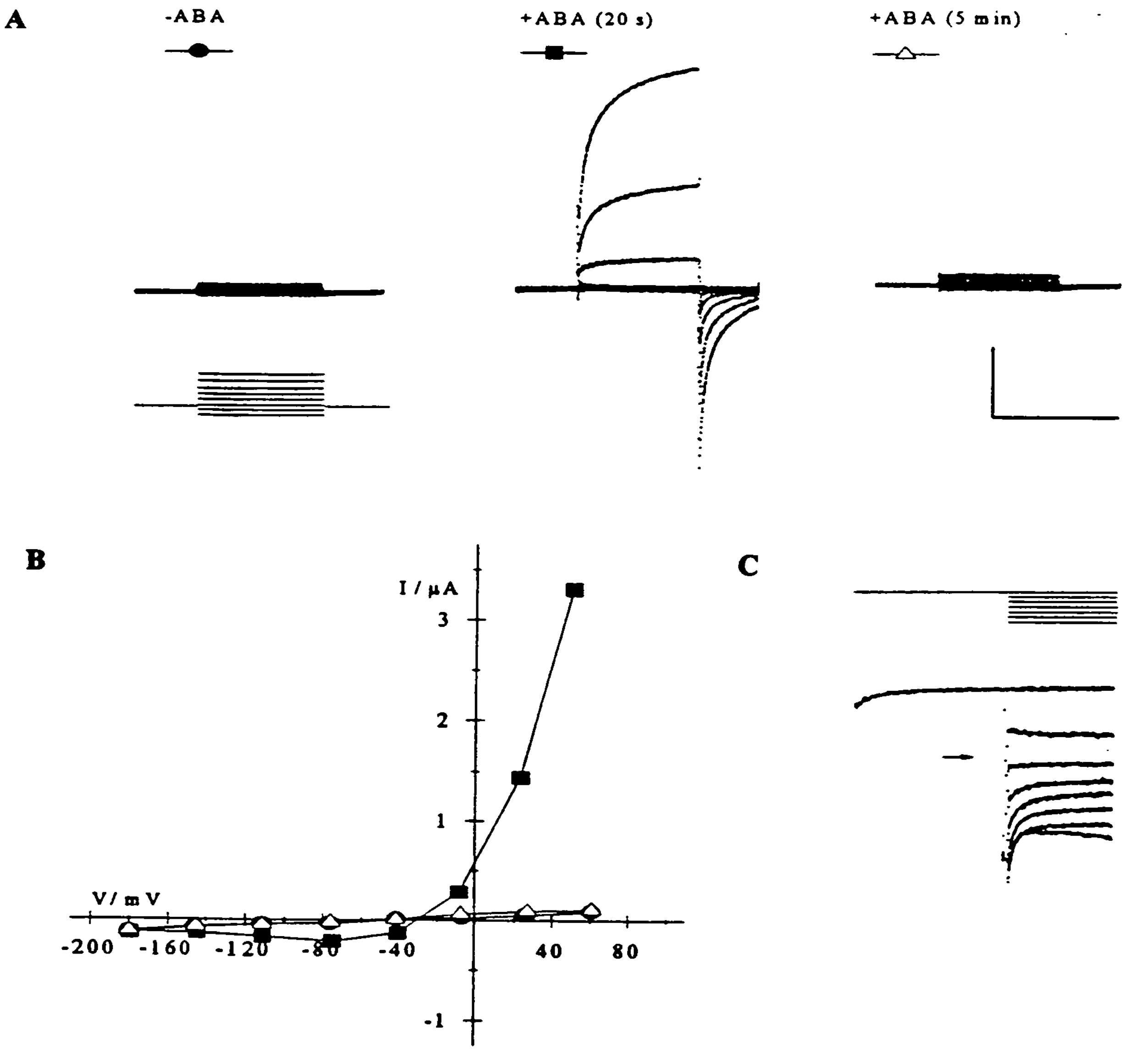


FIG.16

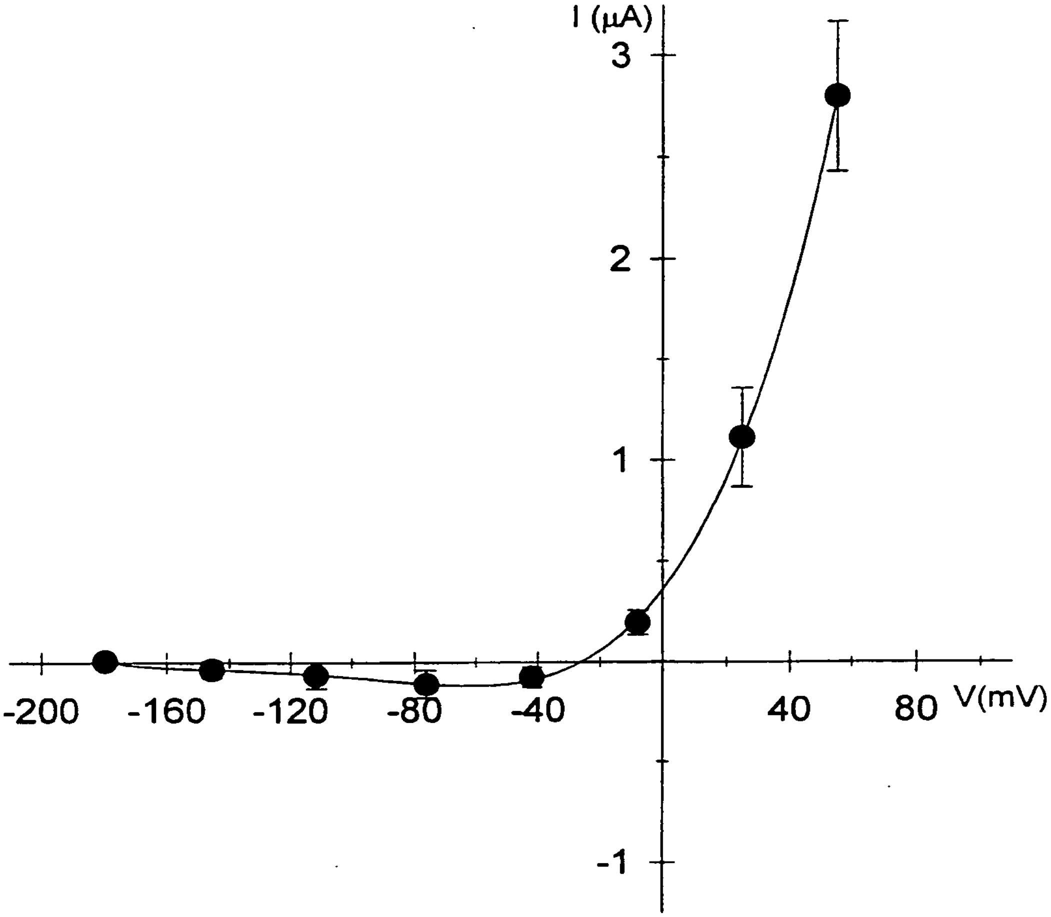


FIG. 17

09509738.032400

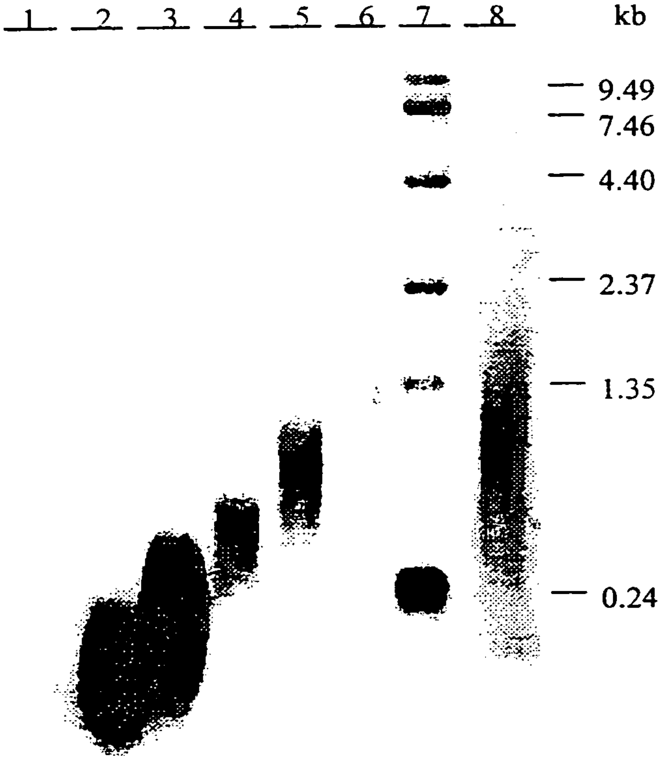

1
2
3
4
5
6
7
8
kb
9.49
7.46
4.40
2.37
1.35
0.24

FIG. 18

09/509738

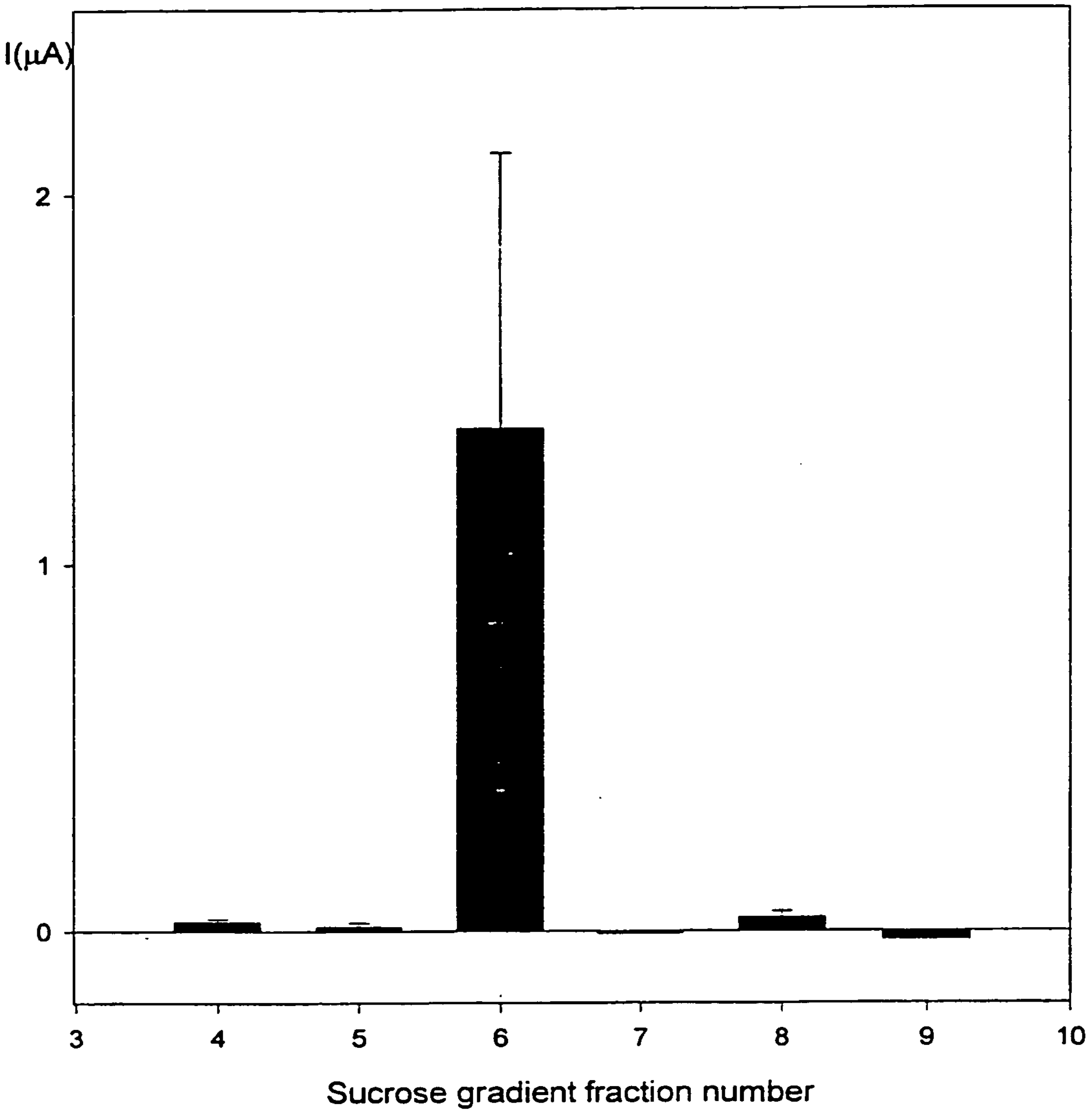


FIG. 19

09509738.032500

09509738.052400

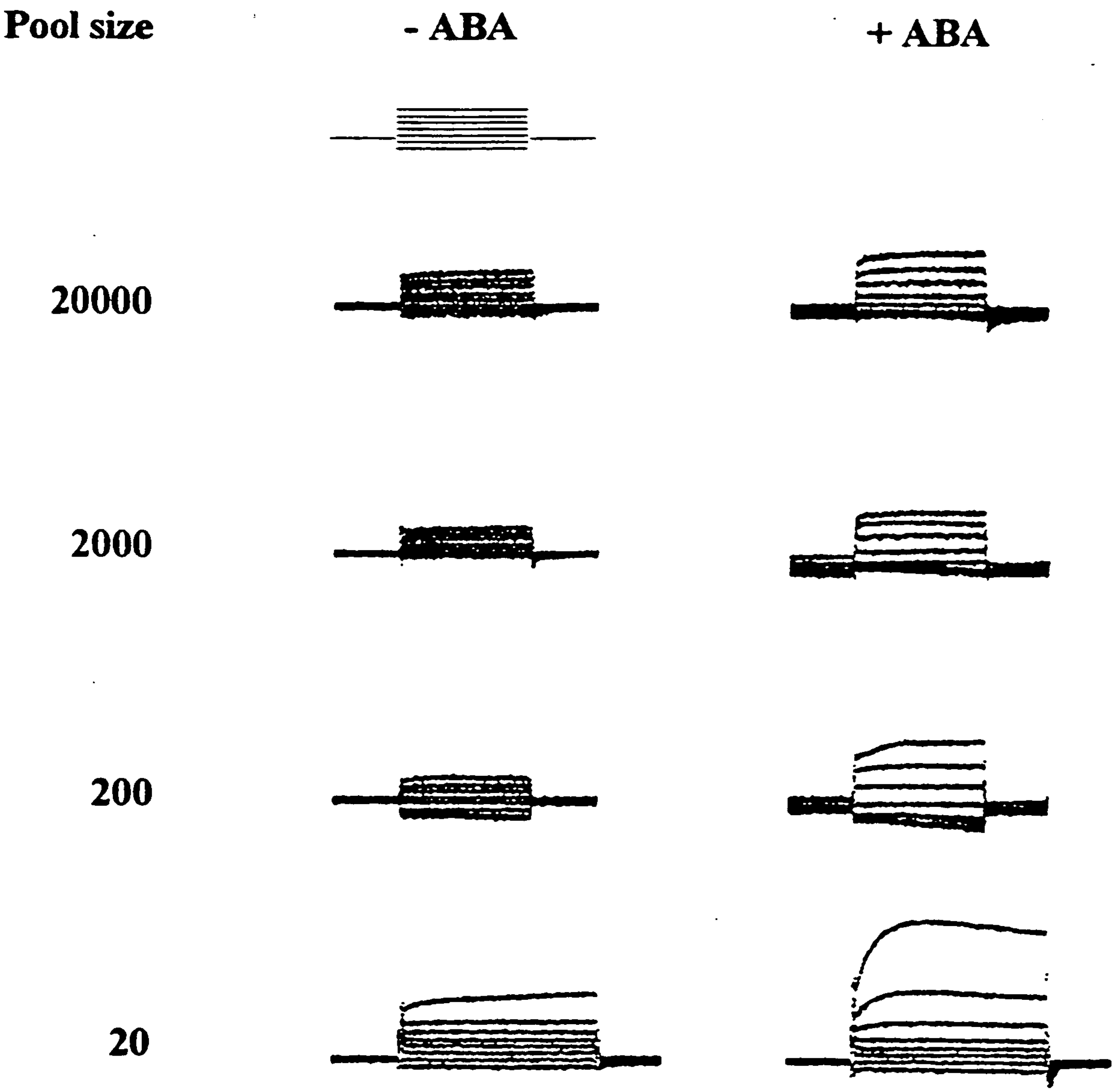


FIG. 20

09509738.052400

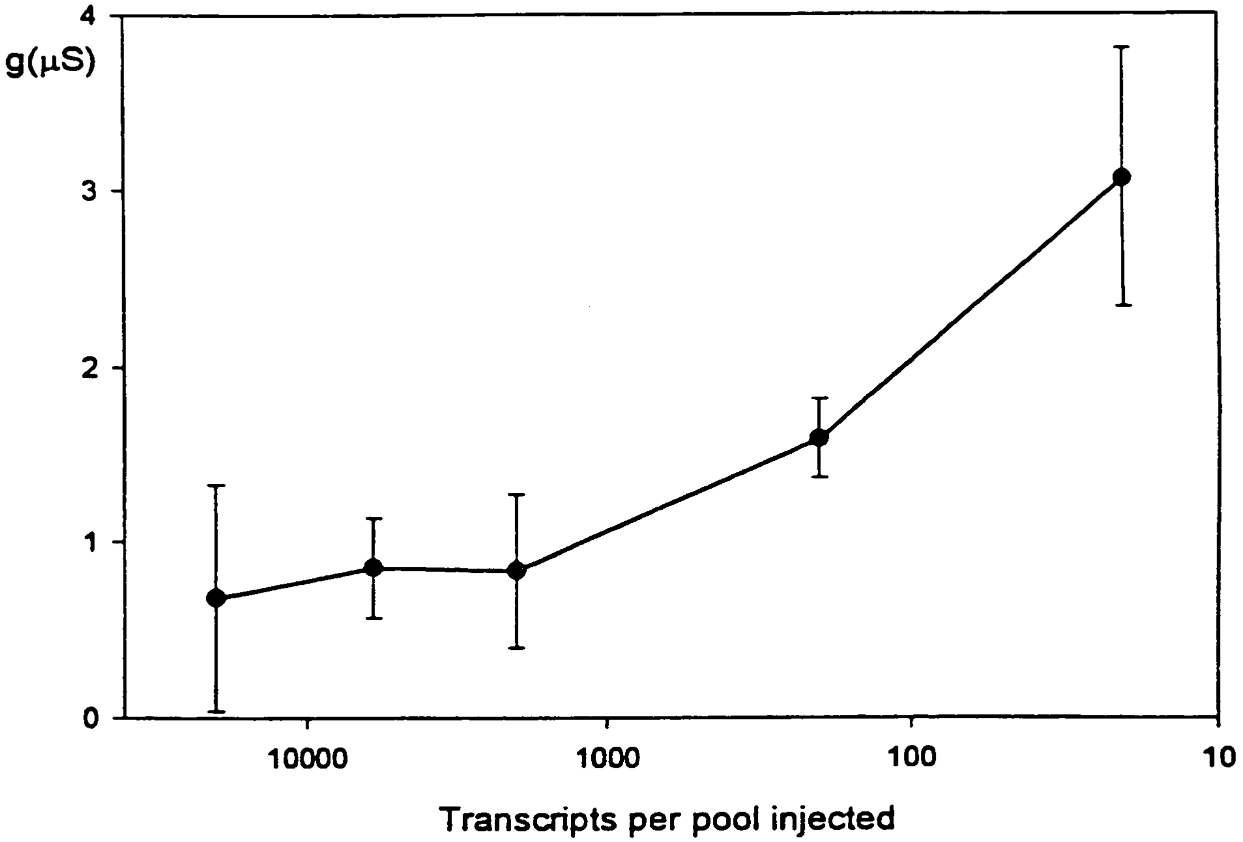


FIG. 21

09/509738

09509738.052500

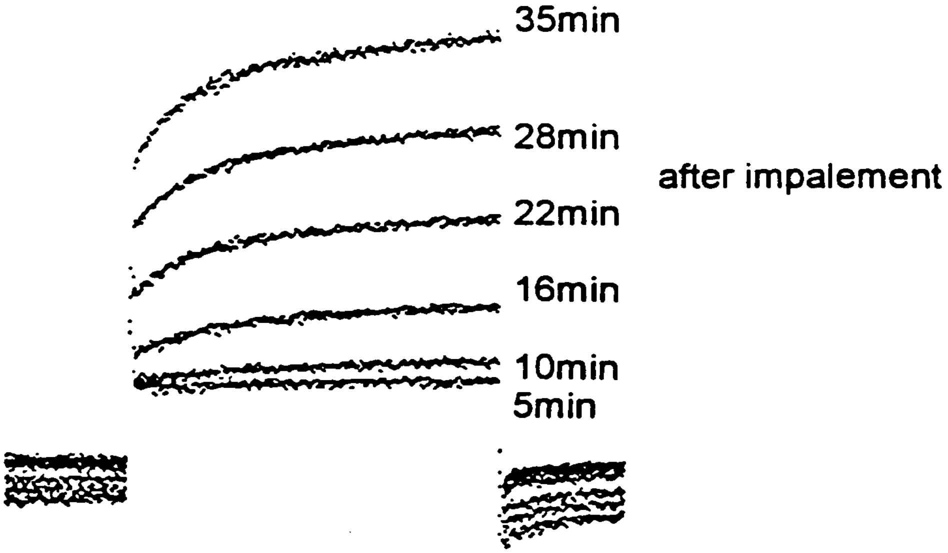


FIG. 22

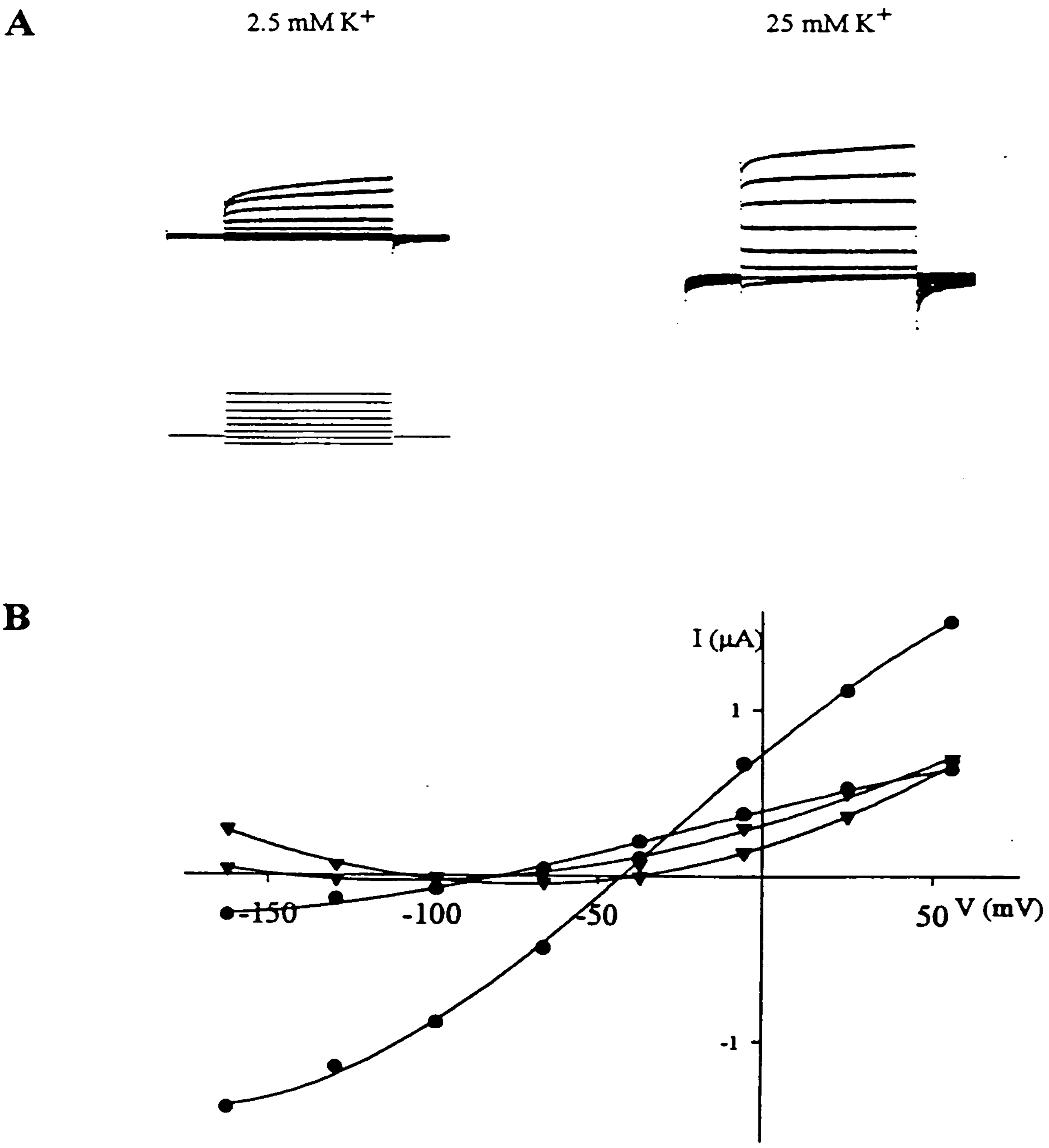


FIG.23

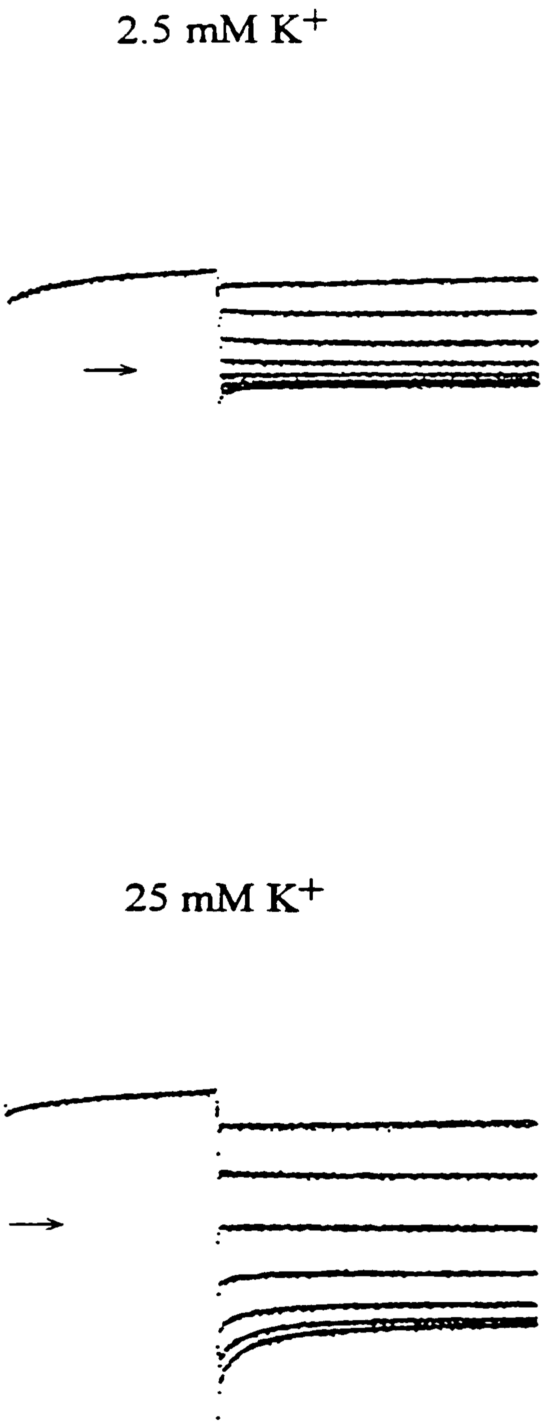


FIG. 24

09509738 .032500

09509738.052400

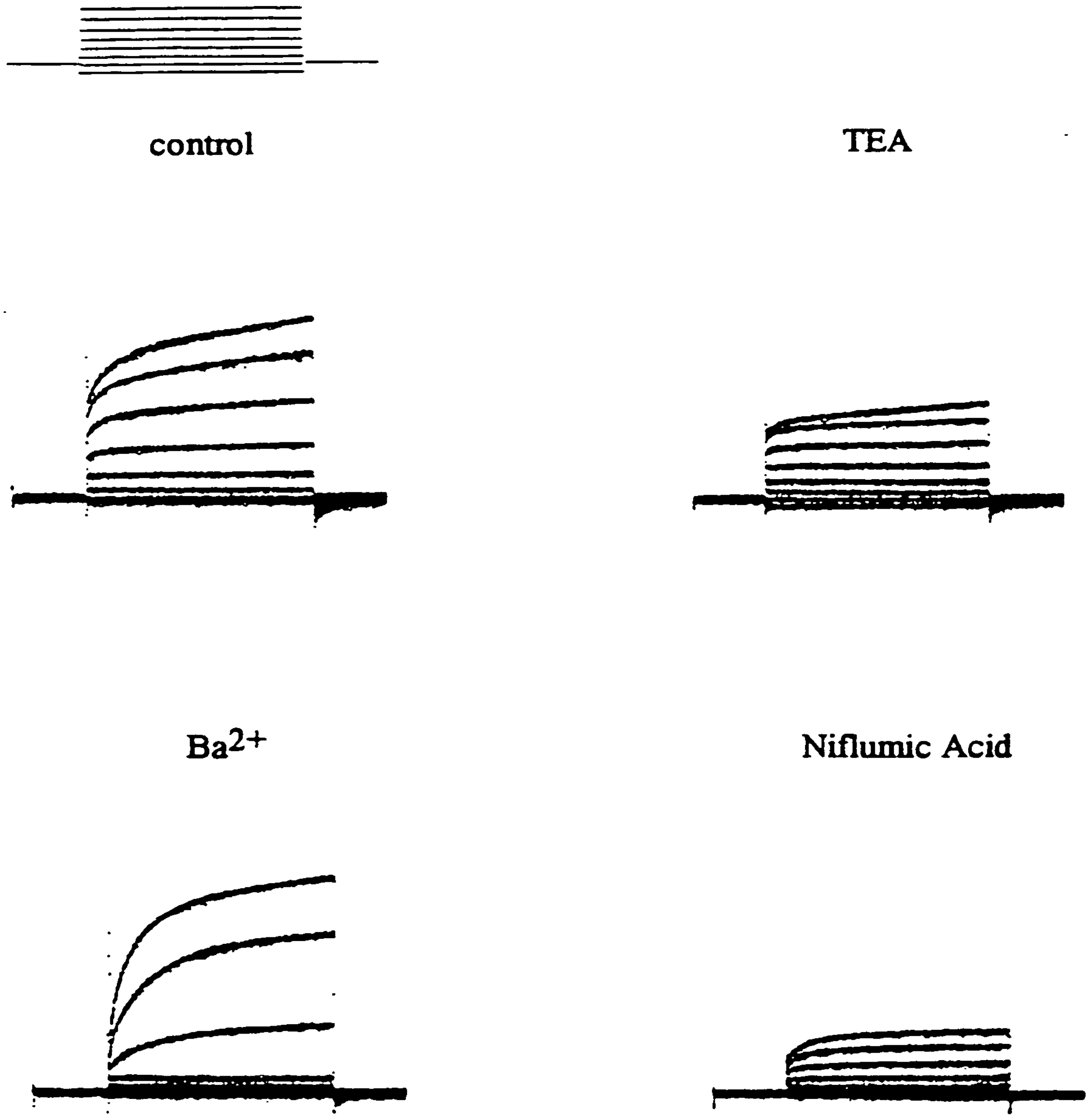


FIG. 25

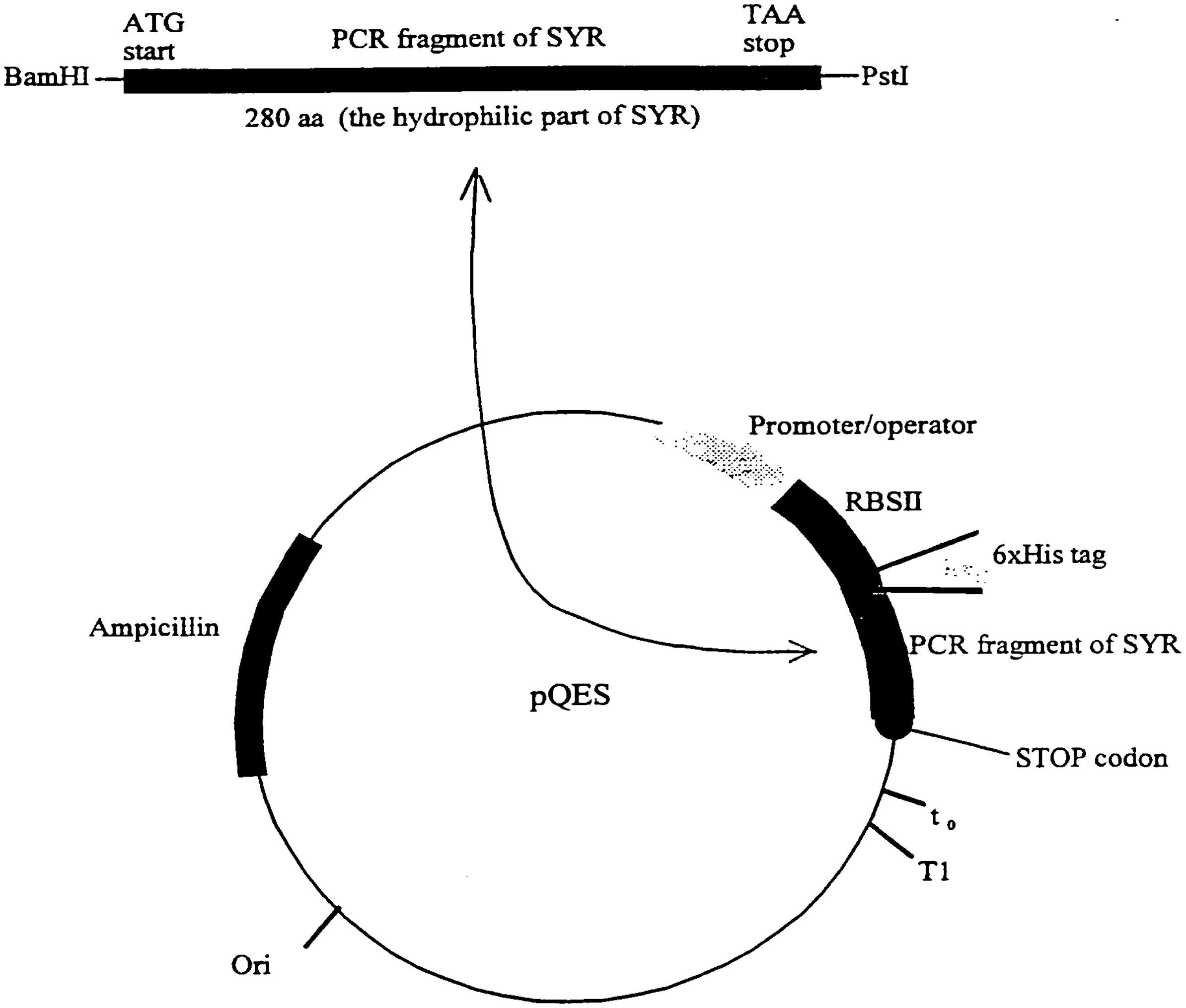

ATG
start
PCR fragment of SYR
TAA
stop
BamHI
PstI
280 aa (the hydrophilic part of SYR)
Promoter/operator
RBSII
6xHis tag
PCR fragment of SYR
STOP codon
t₀
T1
Ampicillin
pQES
Ori

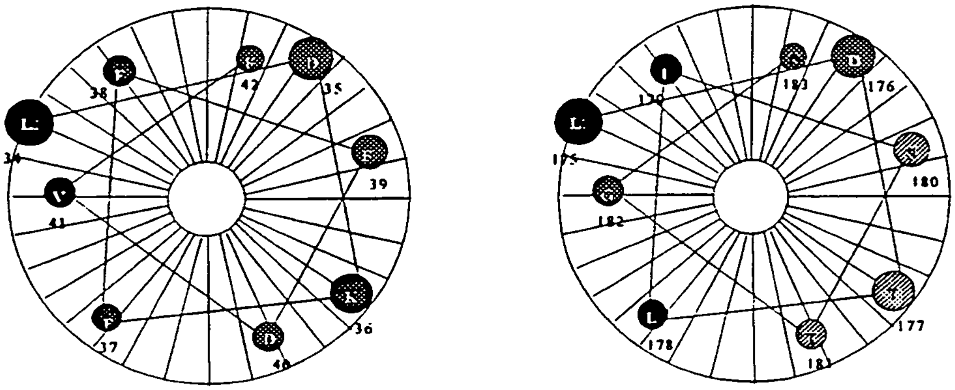
FIG. 26

FIG. 27

A.

LQVARK	SYR-Nt
TKKALK	syntaxin 1A-DRO
TKKAVK	syntaxin 1A-RAT, syntaxin 1A-HUM
TDKAVK	SSO1-yeast
TNKAVK	SSO2-yeast

B.


L
34
F
38
42
D
35
E
39
V
41
K
36
F
37
D
40
I
179
183
D
176
L
175
180
G
182
L
178
177
181

X1

X2

09509738 . 032500

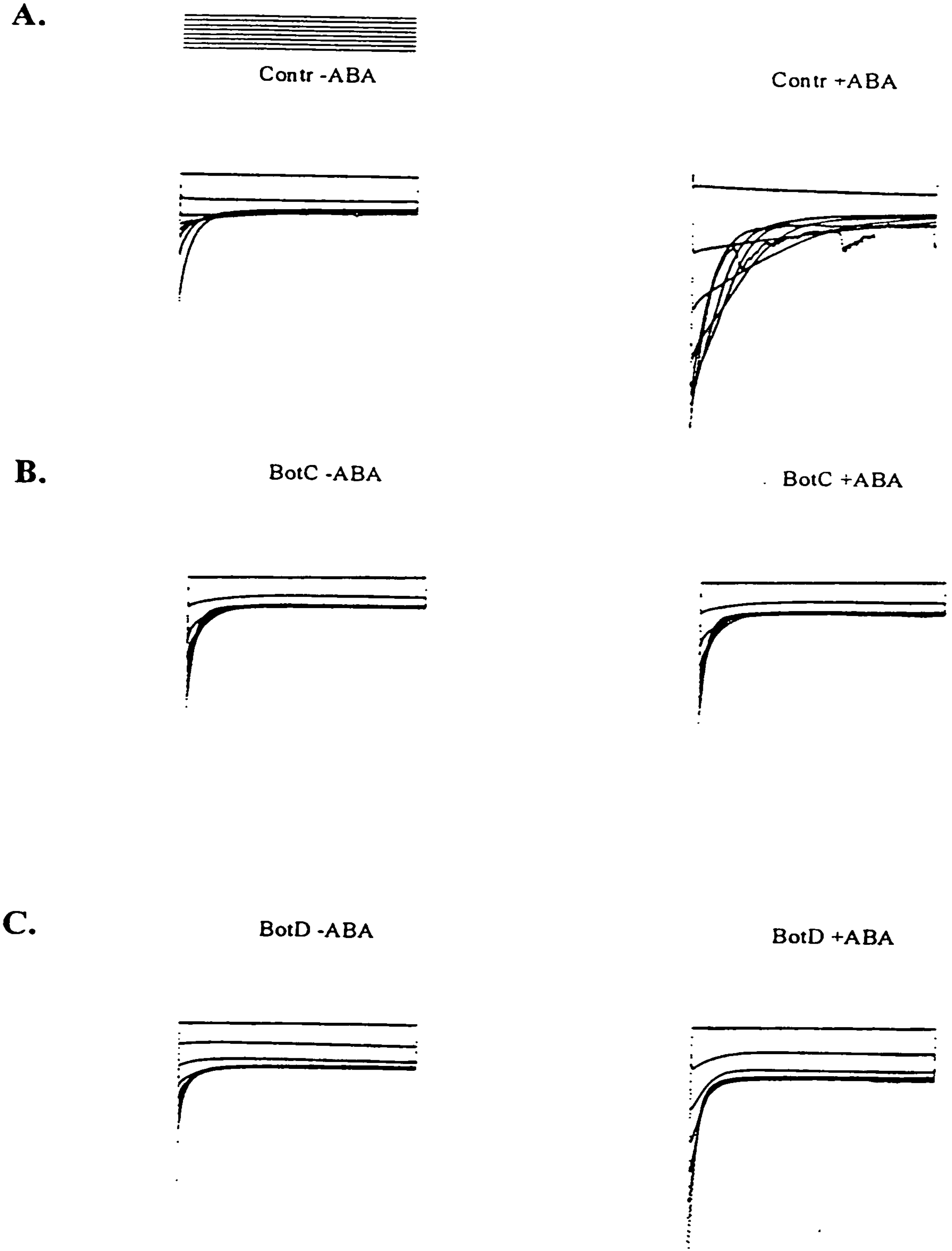


FIG. 28

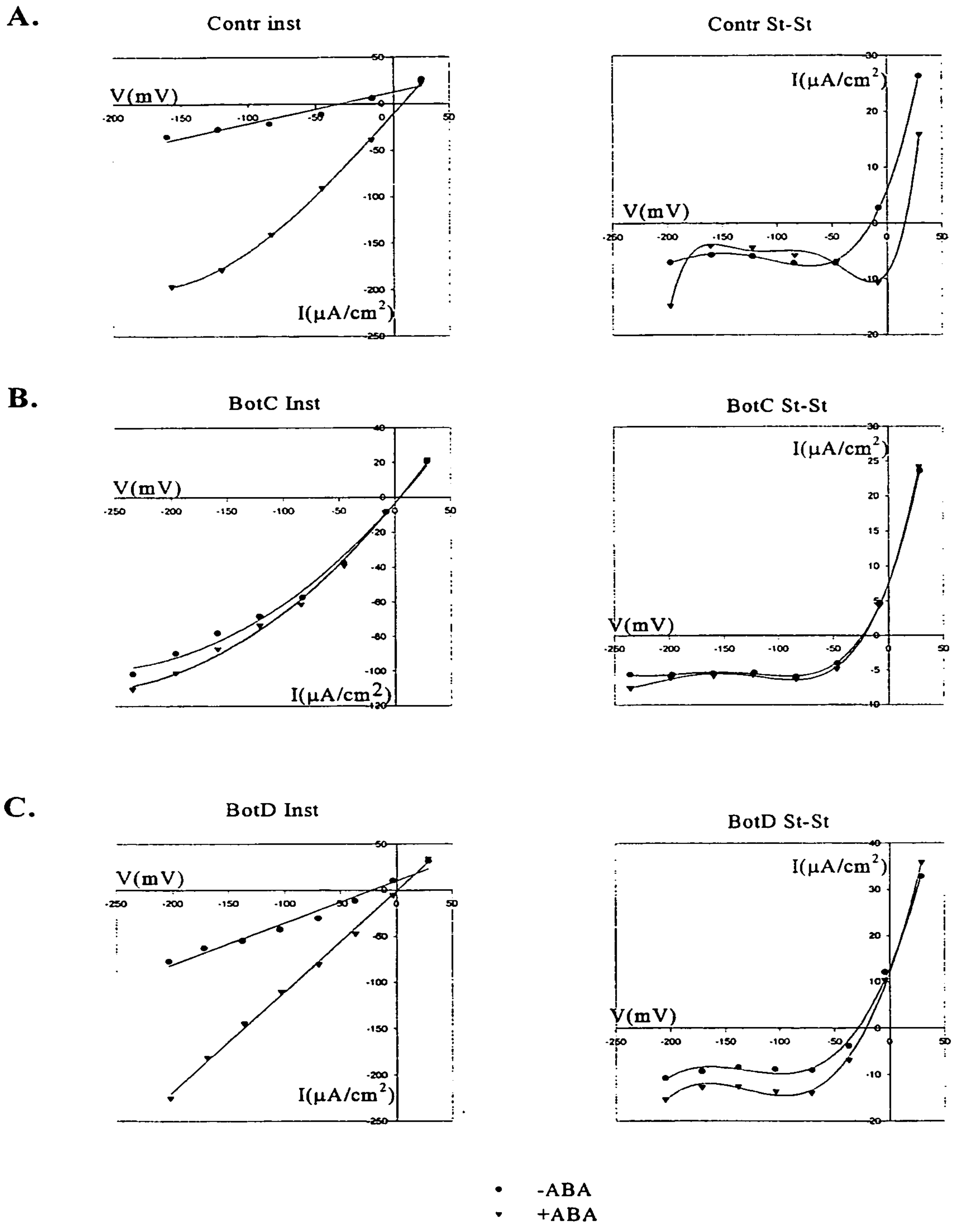

A.
Contr inst
V(mV)
I(μA/cm²)
-200
-150
-100
-50
0
50
-250
Contr St-St
I(μA/cm²)
V(mV)
30
20
10
-10
-20
B.
BotC Inst
V(mV)
I(μA/cm²)
40
20
-20
-40
-60
-80
-100
-120
BotC St-St
I(μA/cm²)
V(mV)
25
15
5
-5
-10
C.
BotD Inst
V(mV)
I(μA/cm²)
BotD St-St
I(μA/cm²)
V(mV)
40
• -ABA
▾ +ABA

FIG. 29

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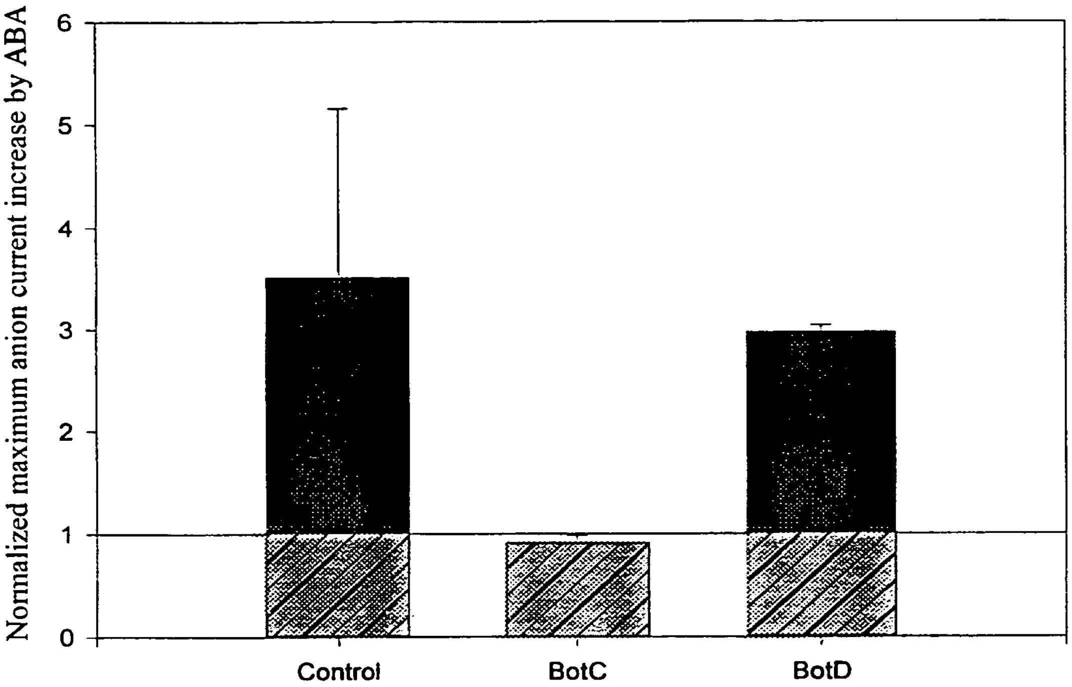
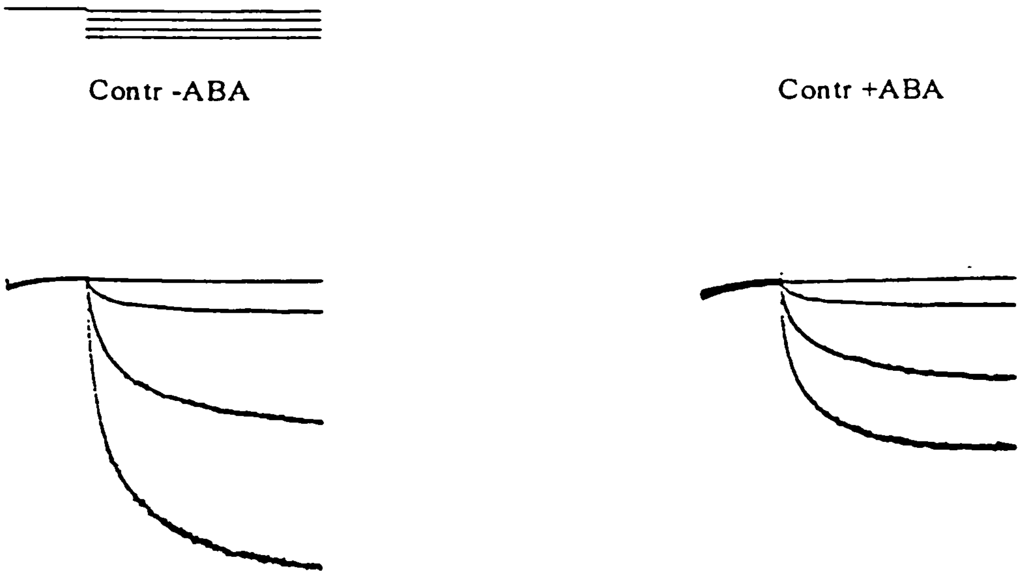


FIG. 30

APPROVED BY DRAFTSMAN
O.G. FIG.
CLASS SUBCLASS

A.



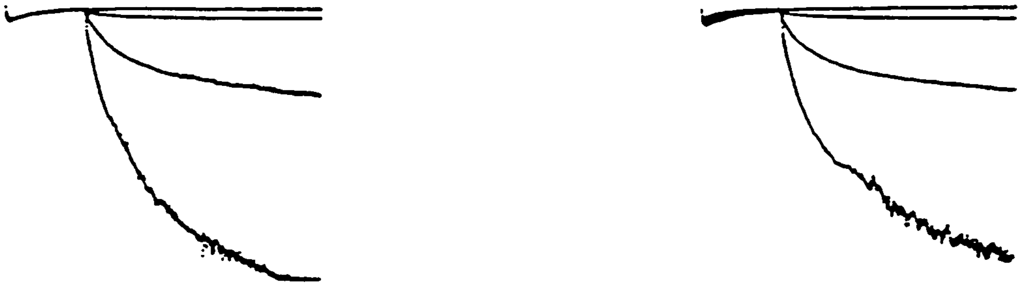
Contr -ABA

Contr +ABA

B.

BotC -ABA

BotC +ABA



C.

BotD -ABA

BotD +ABA

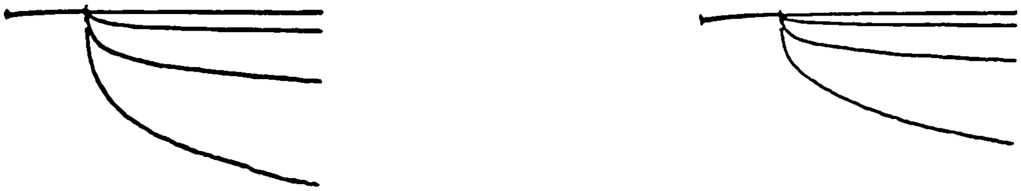


FIG. 31

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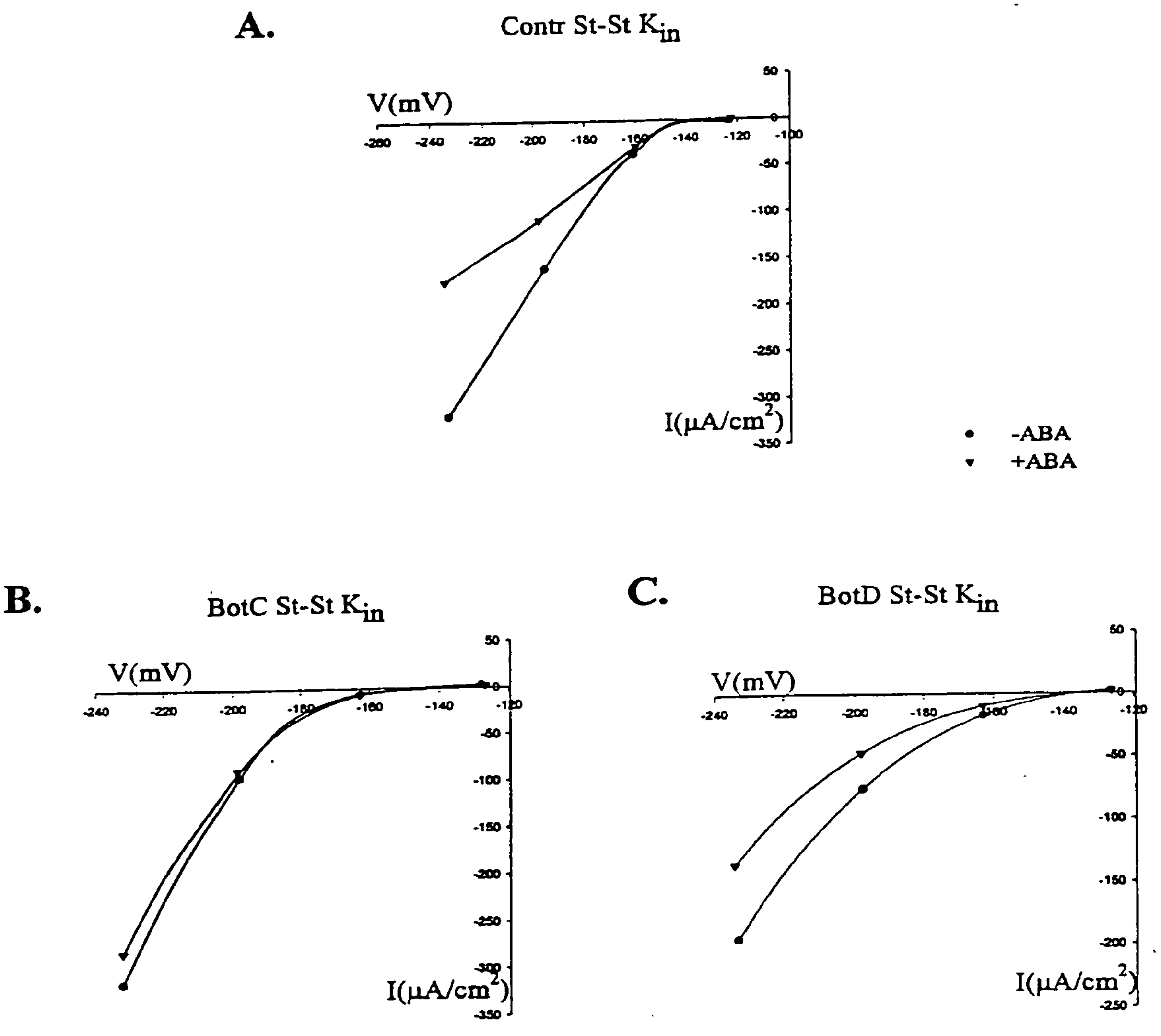
A.
Contr St-St K_{in}
V(mV)
-260
-240
-220
-200
-180
-160
-140
-120
-100
50
0
-50
-100
-150
-200
-250
-300
-350
I(μA/cm²)
● -ABA
▾ +ABA
B.
BotC St-St K_{in}
V(mV)
-240
-220
-200
-180
-160
-140
-120
50
0
-50
-100
-150
-200
-250
-300
-350
I(μA/cm²)
C.
BotD St-St K_{in}
V(mV)
-240
-220
-200
-180
-160
-140
-120
50
0
-50
-100
-150
-200
-250
I(μA/cm²)

FIG. 32

09509738 .042500

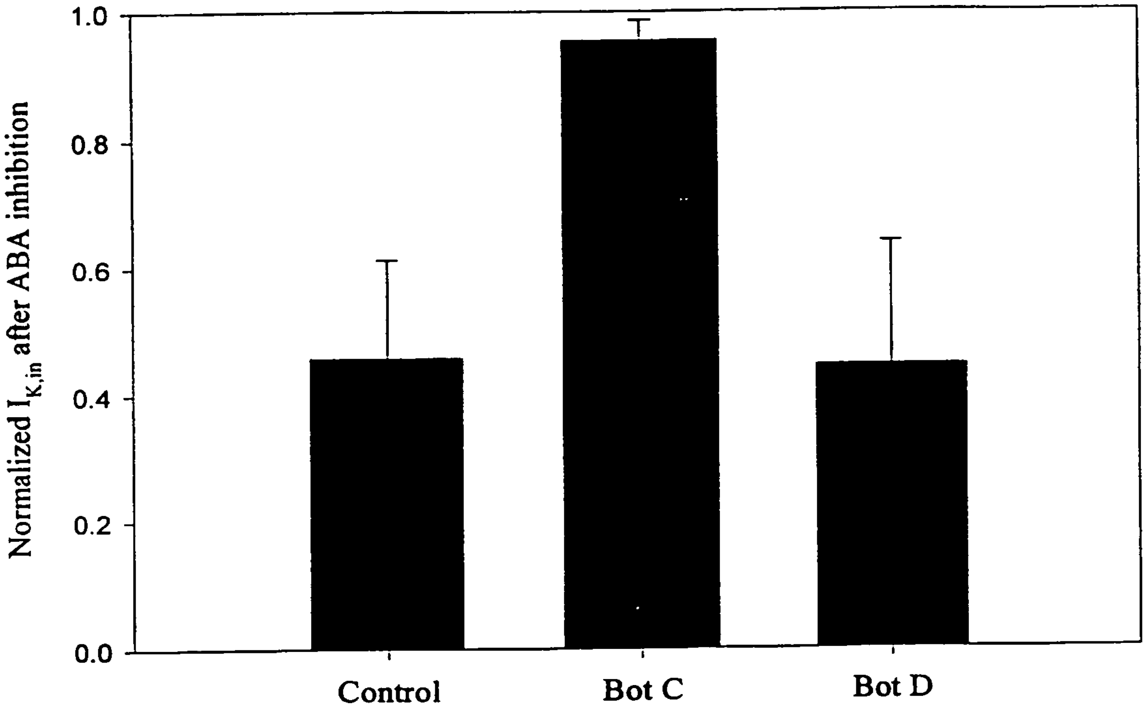

FIG. 33

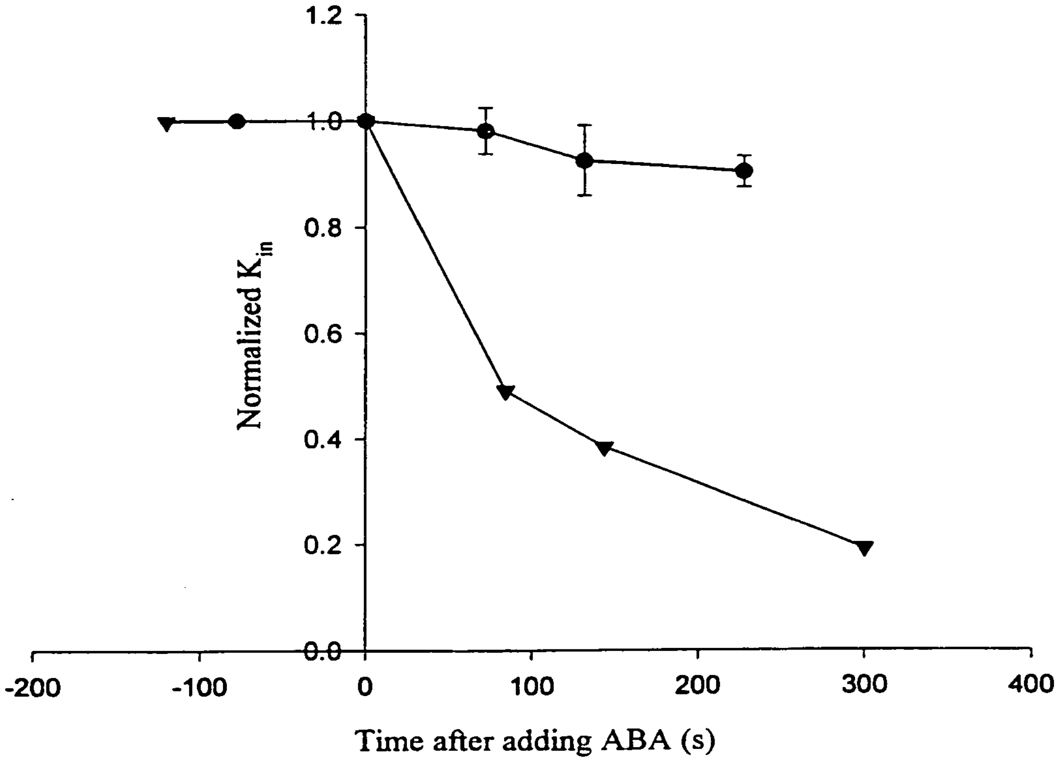
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normalized K_{in}
-200
-100
0
100
200
300
400
Time after adding ABA (s)

FIG. 34

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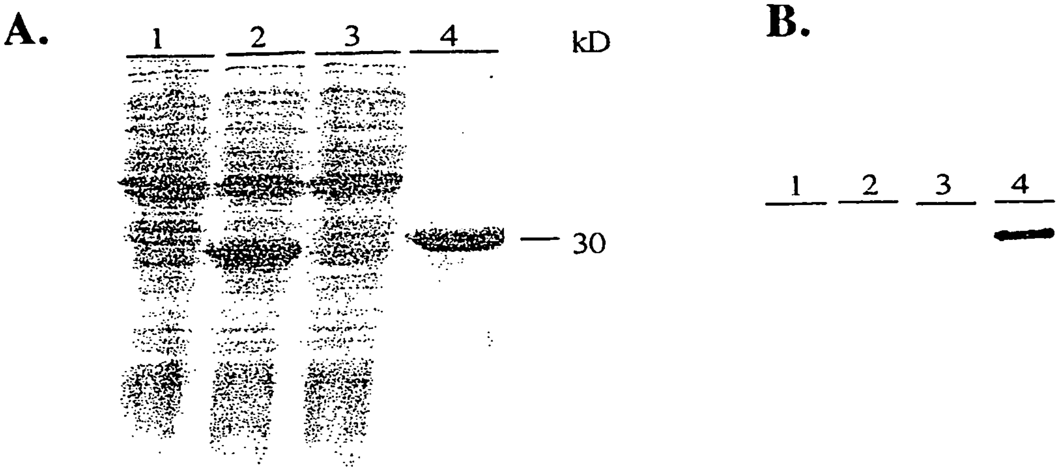
FIG. 35


A.
1
2
3
4
kD
30
B.
1
2
3
4

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APPROVED BY DRAFTSMAN | O.G. FIG. | CLASS | SUBCLASS

WO 99/16880

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PCT/GB98/02937

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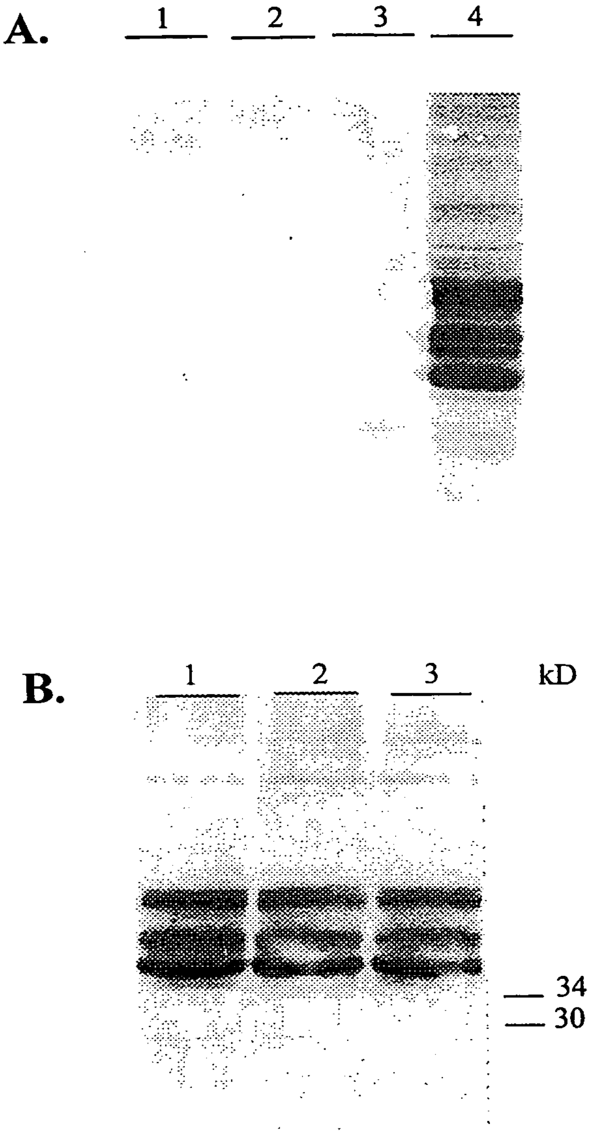
A.
1
2
3
4
B.
1
2
3
kD
34
30

FIG. 36

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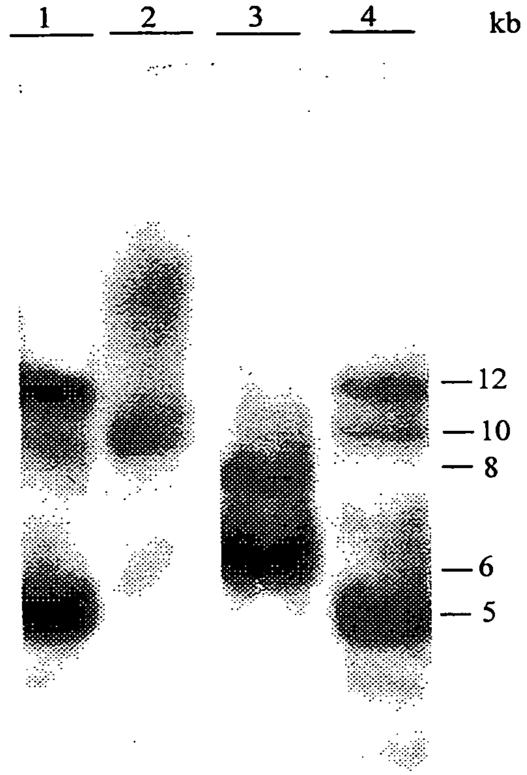
1
2
3
4
kb
12
10
8
6
5

FIG. 37

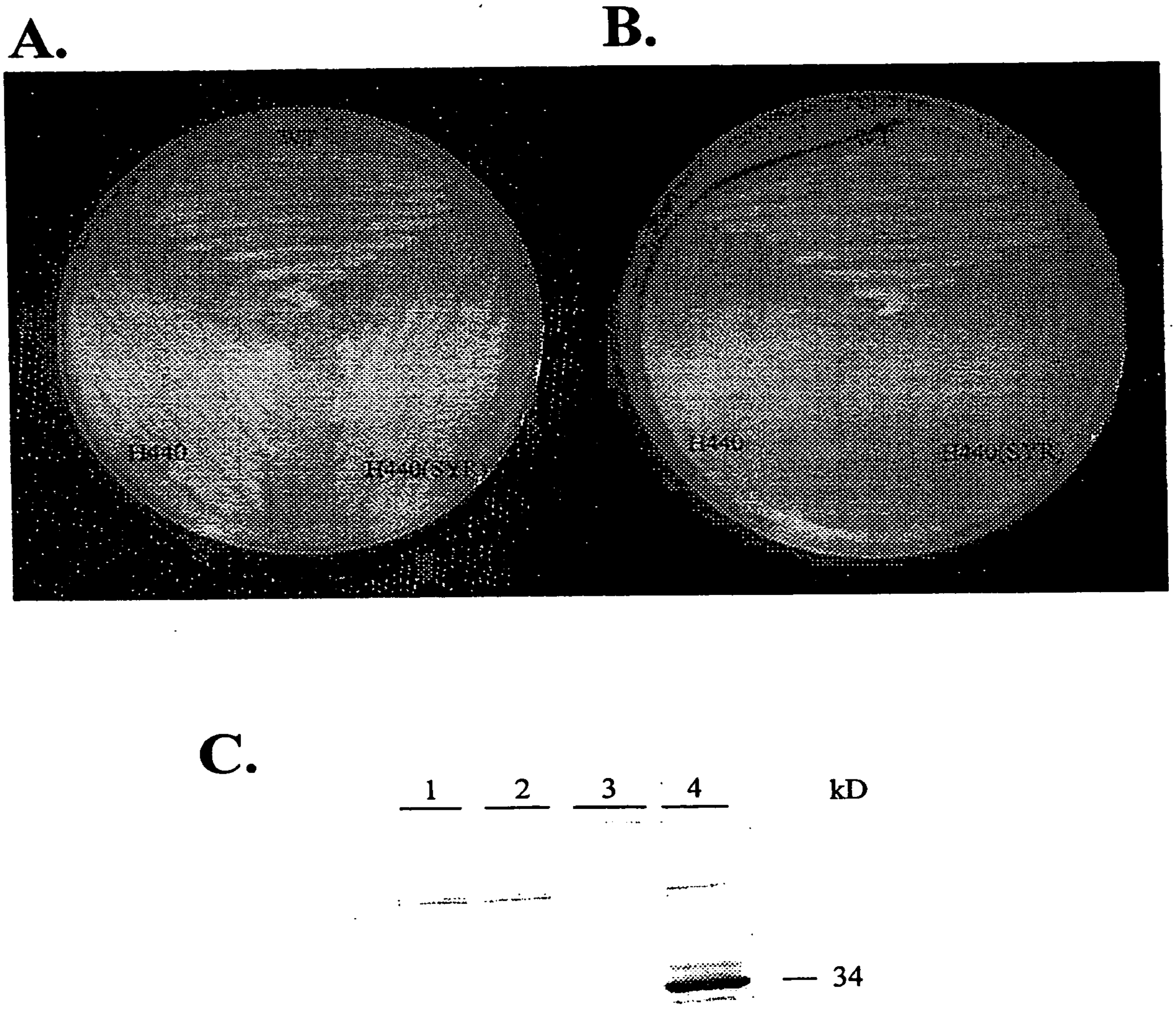
A.
B.
H440
H440(SYT)
H440
H440(SYT
C.
1
2
3
4
kD
34

FIG. 38

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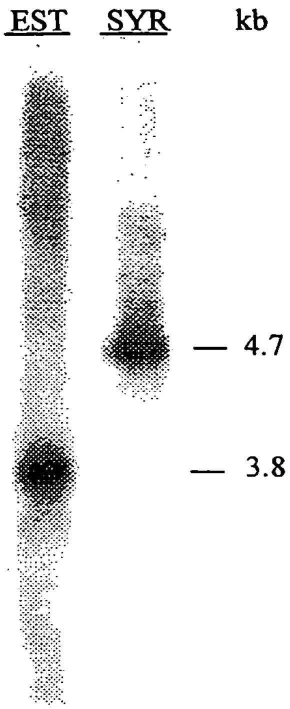
EST
SYR
kb
4.7
3.8

FIG. 39

```
TTTAGATTTACTCTTATATTAGTTTGTTTGTTTAATTGGACGGTTGTTATATCTTTTTCTTA
ATATGAGATTTATGTCGTTATTAATGTTTTTCTCTTGAGGGTTCATAAAGAGATTTATCGTG
TACCTGGGGGTAGGTCAAATGAGAAGGGGTGTAATTTTTGTTTTTTTTTTAGGTTTTATTGT
GTTTTATTATTCGTACCGATTTTATTATTTTATATTTTTAAATCTTATAAGTTTTGTAACTT
CCCCAGGTGGTCTTCTGGAAACTGGTATCTGTTTAAGAGTAAAAAAGGTACCGACTTATCTT
TCTTGGTGGTGGTTTTACTACTATTCGTCTTCTTATTATCGTTTTGTTCAGGTAAAAGATCA
CAAGAAGACCACGAAGGAACGGTGAACGTCAACGACTCGTGGGGGAGATTGCTTCCTTAATC
GGGATAAGTGAACCGAGAGTTATAGTAGTTCAACTCGAGGAACTGAAAGTTGGTTTTGTCGG
TACAGGTTCTTATGAACTACGTTAAGAAAGTTTAAGGAGAGGTTAAGGAAGTGTCGAAGTAC
GGAAAGAACTTAAAGGTATTGACATAGGTAGTGAACAGGAGAGGGAACGAGAACTTAACGGA
AAACGTTCTTGCAGAGTGAAACTGGACATCTATACTCACATAGTTCTTGACGAAGTAGTCCT
AAAAGAGGACACTGCCATATTATAGCAGCAACTTGACAAAGGGATATAAGTCTACGGTAGAA
AACGGAATCGAGCAACTTGACTAAGTAACTGAGAACTTCAAAGAAGGAATTAGGCAAGTGTT
GACTTCAAGAGCAGGACAGTCTACTTGAAGGCCCAGGTGTAGGTCCCTCCGAAGCTAAGCGA
CGTAAACTAGACAGATTCCGAAGCTCTGCTTGAAACTACTTAAACCGAAAGAAGTTACGGTA
CCTTTGCAGTAATAGGTACAACCTAGAATCTAGAAATTGCCGAAATCGCAACACTTCTCAGA
ACGAAAAAAGTACCCTTCTAACCTCAACCCTTATCTAAAAGAGCTCCGGAAACTCAAGCAGA
AATTACCGAAGTTGTAGAAGCTTCTTAAACAGCTCTAACTGAGGCGGTCATTACAGAGGGTA
AAGATACCGCACTCTCAGGCTAACTAGCAGTCGAGACTTTGCTCTCTTTCTAGGACTTTTAT
CTAGTAAGTAAAACTCTACCCTAAACC
```

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FIG. 40

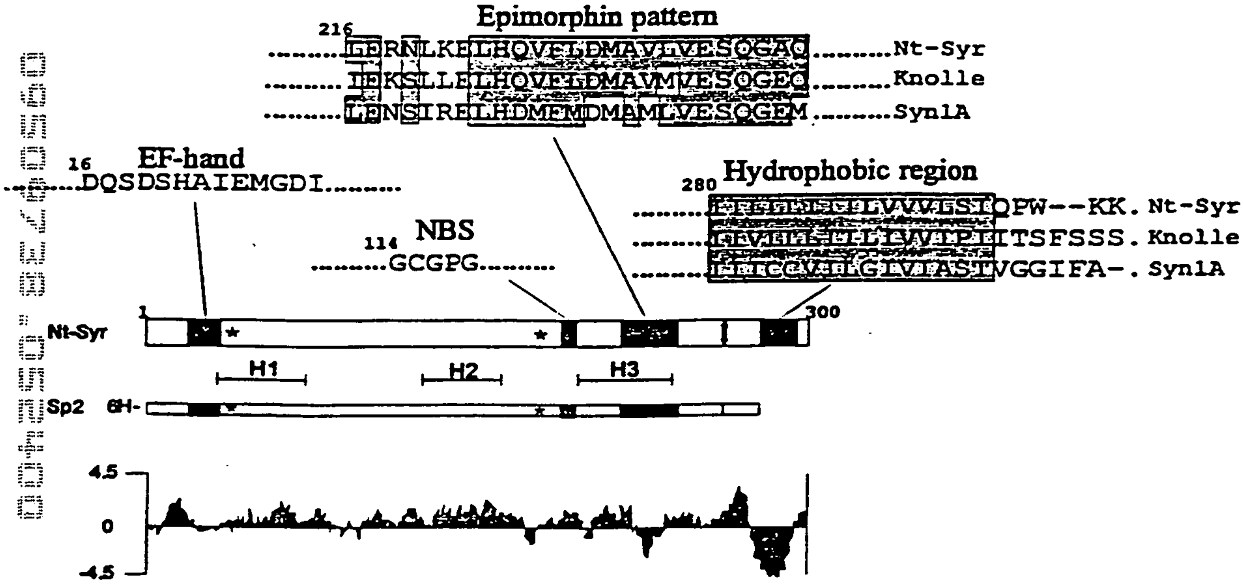

Epimorphin pattern
216
LERNLKELHQVFLDMAVLVESQGAQ Nt-Syr
IEKSLLELHQVFLDMAVMVESQGEQ Knolle
LENSIRELHDMFMDMAMLVESQGEM SynlA
16
EF-hand
DQSDSHAIEMGDI
114
NBS
GCGPG
280
Hydrophobic region
LVVVLGSIQPW--KK. Nt-Syr
TSFSSS. Knolle
VGGIFA-. SynlA
1
300
Nt-Syr
H1
H2
H3
Sp2 6H-
4.5
0
-4.5

FIG. 41

FIG. 42

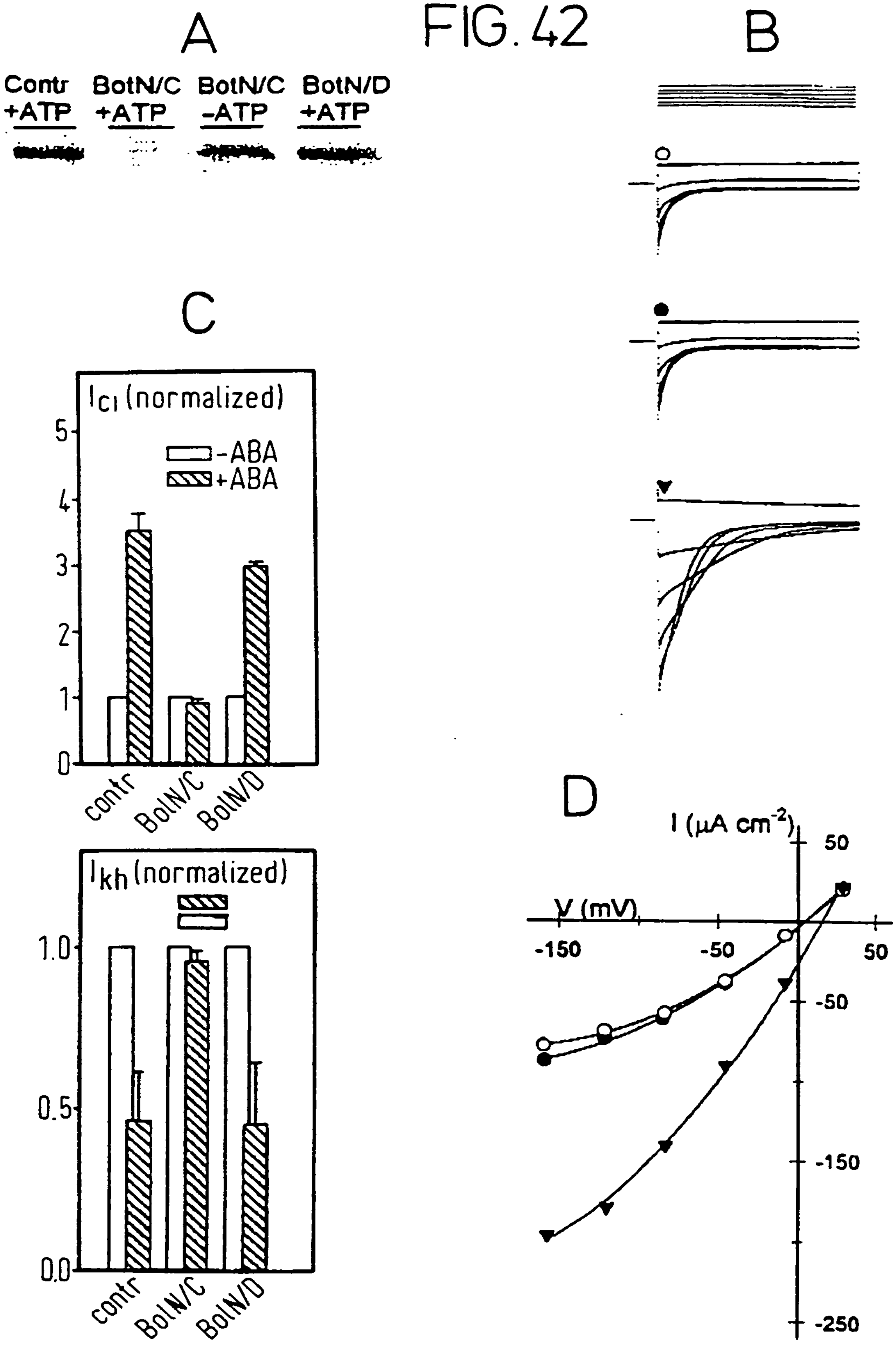
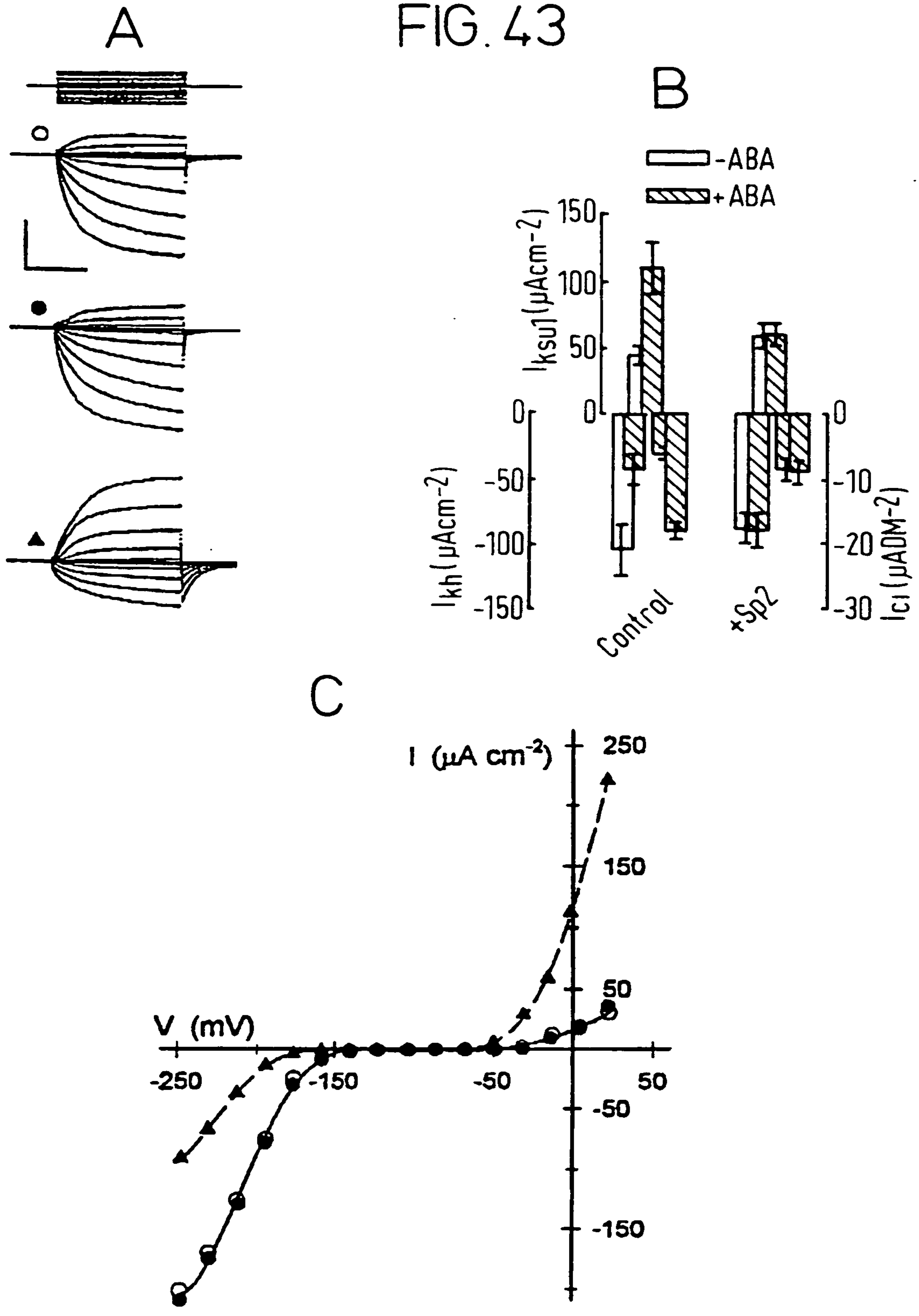

A
Contr +ATP
BotN/C +ATP
BotN/C -ATP
BotN/D +ATP
B
○
●
▼
C
I_{Cl} (normalized)
□ -ABA
▨ +ABA
5
4
3
2
1
0
contr
BotN/C
BotN/D
I_{kh} (normalized)
1.0
0.5
0.0
contr
BotN/C
BotN/D
D
I (μA cm^{-2})
V (mV)
-150
-50
50
50
-50
-150
-250

FIG. 43



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